Quality Assessment of Domestic Harvested Rainwater in the Peri-Urban Region of Kleinmond, Western Cape and the Optimisation of Point-of-Use Treatment Systems

By

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Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and have not previously in its entirety or in part submitted it at any university for a degree.

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Summary

Domestic rainwater harvesting (DRWH) refers to the collection and storage of rainwater for domestic purposes and in an effort to achieve the Millennium Development Goals (MGD), the South African government has started an initiative where DRWH tanks are financed in sustainable housing schemes in an aim to provide an additional water source directly to households. Although many provinces, including parts of the Eastern Cape and KwaZulu Natal, have been using harvested rainwater as a potable water source, there are a limited number of studies indicating the quality of harvested rainwater in South Africa. However, many studies, internationally, have indicated that while the practice of harvesting rainwater is gaining popularity, rainwater quality is not within potable standards (Chapter 1).

During the first phase of the study, rainwater samples were collected from the Kleinmond Housing Scheme (Western Cape, South Africa). From a cluster of 411 houses, the DRWH tanks connected to 29 houses were selected for monitoring the microbial and physico-chemical properties of harvested rainwater. Drinking water guidelines stipulated by SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) were used throughout the study to monitor the quality of rainwater. Eight sampling sessions were then conducted from March to August 2012, during a high and low rainfall period. Overall, the physico-chemical parameters of the rainwater samples were within the respective drinking water guidelines. However, the microbiological analysis verified results obtained in international studies, and showed that the indicator bacteria numbers present in the DRWH samples exceeded the stipulated guidelines (Chapter 2 and 3). Species specific primers were also used to routinely screen for the virulent genes, *aggR, stx, eae* and *ipaH* found in Enteroaggregative *E. coli* (EAEC), Enterohaemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC) and Enteroinvasive *E. coli* (EIEC), respectively, in the rainwater samples. The virulent pathogenic *E. coli* genes were then detected in 3% (EPEC and EHEC) and 16% (EAEC) of the 80 rainwater samples collected routinely during the sampling period from ten DRWH tanks (Chapter 3). Bacterial isolates selected during the high rainfall period (June to August 2012), as well as PCR assays performed on total genomic DNA extraction from the rainwater samples, confirmed the presence of numerous pathogenic bacteria including *Legionella* spp. *Klebsiella* spp. and *Shigella* spp. *Yersinia* spp. were also isolated and detected for the first time in DRWH tanks (Chapter 4).

Based on the results obtained in the first phase and as many studies have indicated the poor quality of rainwater, the second phase of the project was aimed at designing and monitoring point of use treatment systems. Three polyethylene DRWH tanks (2000 L) were installed at the Welgevallen

Experimental farm, Stellenbosch University, South Africa. Various treatment systems, such as activated carbon and slow sand filtration, solar pasteurization and a combined activated carbon/PVA nanofibre filtration column, were then intermittently connected to the three DRWH tanks during the high rainfall period (June to October 2013). Results for slow sand filtration and activated carbon filters indicated that the biological layer that had developed on the filtration media had not matured and for this reason chemical and microbial parameters were not reduced to within drinking water guidelines. A polyvinyl (alcohol) (PVA) nanofibre membrane without activated carbon in a column filtration system was analysed and results indicated that this system was also not effective in reducing the microbial numbers to within drinking water guidelines. Lastly, by utilising a PVA nanofibre membrane with activated carbon in a column filtration system, one litre of potable water was produced and all heterotrophic bacteria, *E. coli* and total coliform counts were reduced to zero and were within drinking water guidelines (Chapter 5).

For the solar pasteurization system (Chapter 6), at treatment temperatures of greater than 72°C, all heterotrophic bacteria, *E. coli* and total coliforms were reduced to zero and were within drinking water guidelines. However, PCR assays confirmed the presence of *Yersinia* spp., *Legionella* spp., and *Pseudomonas* spp., at temperatures greater than 72°C. Results for chemical analysis also indicated all cations were within the international and national drinking water guidelines, with the exception of iron, aluminium, lead and nickel, which were detected in the pasteurized rainwater samples and were above the respective guidelines. It is hypothesised that these elements could have leached from the stainless steel storage tanks of the pasteurization system and it is therefore recommended that the storage tank of the pasteurization system be manufactured from an alternative material, such as a high grade polymeric material, which is able to withstand the high temperatures yet will not negatively influence the quality of harvested rainwater.

Opsomming

Reënwater versameling vir huishoudelike gebruik verwys na die versameling en berging van reënwater vir huishoudelike doeleindes. In 'n poging om die Millennium Ontwikkelingsdoelwitte (MOD) te bereik het die Suid-Afrikaanse regering ‘n inisiatief begin om finansiële bystand aan huishoudings te verleen vir die implementering van reënwater tenke, in ‘n poging om ‘n addisionele water bron direk aan huishoudings te verskaf. Hoewel baie provinsies, insluitend dele van die Oos-Kaap en KwaZulu-Natal, reënwater gebruik as ‘n drinkbare water bron, is daar 'n beperkte aantal studies beskikbaar oor die gehalte van versamelde reënwater in Suid-Afrika. Baie studies internasionaal het egter al aangedui dat, alhoewel die praktyk van die versameling van reënwater besig is om in gewildheid toe te neem, die kwaliteit van reënwater nie binne drinkbare standaarde is nie (Hoofstuk 1).

Tydens die eerste fase van die studie is reënwater monsters geneem van die Kleinmond Behuisings Skema (Wes Kaap, Suid Afrika). Van ‘n kompleks van 411 huise, is reënwater tenke van 29 huise geselekteer vir die monitering van die mikrobiese en fisiese-chemiese eienskappe van versamelde reënwater. Drinkwater riglyne soos gestipuleer deur SANS 241 (2005), DWAF (1996), AWDG (NHMRC en NRMMC, 2011) en WHO (2011) was regdeur die studie gebruik om die kwaliteit van reënwater te monitor. Reënwater monsters is tydens agt geleenthede geneem vanaf Maart tot Augustus 2012, tydens die hoë en lae rëenval periode. Oor die algemeen was die fisiese-chemiese parameters van die reënwater monsters binne die onderskeie riglyne vir drinkwater. Mikrobiese analises het egter die resultate van internasionale studies bevestig en het aangedui dat die getal indikator bakterieë teenwoordig in the reënwater die gestipuleerde riglyne oorskry (Hoofstuk 2 en 3). Spesies spesifieke inleiers was gebruik om die virulensie gene *aggR*, *stx*, *eae* en *ipaH*, teenwoordig in onderskeidelik Entero-aggregatiewe *E. coli* (EAEC), Entero- hemorragiese *E. coli* (EHEC), Entero-patogeniese *E. coli* (EPEC) and Entero-indringende *E. coli* (EIEC), in die reënwater monsters op te spoor. Die virulente pathogeniese *E. coli* gene was in 3% (EPEC en EHEC) en 16% (EAEC) van die 80 reënwater monsters, geneem van 10 reënwater tenke tydens die studie, waargeneem (Hoofstuk 3). Bakteriese isolate geselekteer tydens die hoë reenval periode (Junie tot Augustus 2012), so wel as PKR (polymerase ketting reaksie) ontledings uitgevoer op genomiese DNS wat van die reënwater monsters ge-ekstraheer was, het die teenwoordingheid van verskeie patogeniese bakterieë, insluitend *Legionella* spp., *Klebsiella* spp. en *Shigella* spp., bevestig. Dit is ook die eerste keer wat *Yersinia* spp. in versamelde reënwater waargeneem is (Hoofstuk 4).

Die doel van die tweede fase van die projek was om water behandeling sisteme by die punt van gebuik te ontwerp en te monitor aangesien die resultate wat gedurende die eerste fase verkry is, sowel as vele ander studies, aangedui het dat die kwaliteit van versamelde reënwater swak is. Drie poli-etileen reënwater tenke (2000 L) was geïnstalleer op die Welgevallen Eksperimentele plaas van die Universiteit van Stellenbosch, Suid Afrika. Verskeie water behandeling sisteme, soos geaktiveerde koolstof en stadige sand filters, sonkrag pasteurisasie en ‘n gekombineerde geaktiveerde koolstof/PVA nanovesel filtrasie kolom, was met tye gekonnekteer aan verskeie reënwater tenke gedurende die hoë reënval periode (Junie to Oktober 2013). Resultate van die stadige sand en geaktiveerde koolstof filters het aangedui die biologiese laag nog nie ver genoeg ontwikkeld het nie en daarom was die chemiese en mikrobiese parameters nie verminder to binne die riglyne vir drinkwater nie. ‘n Polyviniel (alkohol) nanovesel membraan sonder geaktiveerde koolstof binne ‘n kolom filtrasie sisteem, was geanaliseer en resultate het aangedui dat hierdie sisteem ook nie die mikrobiese getalle verminder het tot drinkwater standaarde nie. Laastens, deur gebruik te maak van ‘n PVA nanovesel membraan met geaktiveerde koolstof in ‘n kolom filtrasie sisteem, was een liter drinkbare water geproduseer met alle heterotrofiese bakterieë, *E. coli* en totale koliform getalle verminder tot nul en was binne die riglyne vir drinkwater (Hoofstuk 5).

Vir die sonkrag pasteurisasie sisteem (Hoofstuk 6), by behandelings temperature van groter as 72°C, was alle heterotrofiese bakterieë, *E. coli* en totale koliform getalle verminder tot nul en was binne die riglyne vir drinkwater. Maar PKR ontledings het die teenwoordigheid van *Yersinia* spp., *Legionella* spp., en *Pseudomonas* spp. by temperature groter as 72°C bevestig. Resultate van die chemiese analises het ook aangedui dat alle kationе binne die drinkwater riglyne was, met die uitsondering van yster, aluminium, lood en nikkel. Hierdie elemente was in die gepasteuriseerde reënwater monsters waargeneem met konsentrasies wat die onderskeie riglyne oorskry het. Dit word gehipoteseer dat hierdie elemente uit die vlekvrye staal van die bergings tenk van die pasteurisasie sisteem kon uitgeloog het. Daarom word dit aanbeveel dat die bergings tenk van die pasteurisasie sisteem geproduseer moet word van ‘n alternatiewe materiaal, soos ‘n hoë graad polimeriese material, wat hoë temperature kan weerstaan maar nie ‘n negatiewe invloed sal hê op die kwaliteit van versamelde reënwater nie.

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Table of Contents

Chapter 1:

Literature Review

Sections of the Literature Review were published in Water, Air and Soil Pollution (2013) Volume 224, 1629

(The literature review is thus compiled in the format of the Water, Air and Soil Pollution Journal and UK spelling is employed)

1.1 Introduction

The Millennium Development Goals (MDG) were officially established by the United Nations (UN) in 2000 (United Nations General Assembly, 2000). These goals were approved by 193 United Nations member states and approximately 23 international organisations, who all agreed that by 2015, the stipulated goals should be achieved. The MDG had three main focus areas, namely, valorising human capital, improving infrastructure and increasing social, economic and political rights. One of the main aims of the MDG was thus to improve infrastructure and significantly increase access to safe drinking water by halving the proportion of people without access to potable water and sanitation by 2015. To date, it is estimated that 768 million people, predominantly in developing countries, rely on substandard drinking water sources while approximately 2.5 billion people lack access to adequate sanitation (WHO/UNICEF, 2013).

In 2008 the UN published the progress of the MDG, with the report highlighting that from 1990 to 2008, internationally, 1.1 billion people residing in urban areas had gained access to an improved water source, and that during the same time frame 723 million people living in rural areas achieved the same goal. In addition the number of people using an improved drinking water source in Sub-Saharan Africa increased from 252 million in 1990 to 492 million people in 2008. However, 11% of the world's population still remains without access to an improved drinking water source and it was therefore predicted that, at the current rate, 605 million people would still lack access to safe drinking water by 2015 (UN, 2012). A highlight, emphasised in the Millennium Development Goals Report (UN, 2011) was that Northern Africa had exceeded their limit of abundant resources and their sustainable limits for water resources, however, Sub-Saharan Africa was approximately 13% below the abundance of water resources limit (25%), with a 57% prediction that this area was rapidly approaching their "water scarcity" limit (UN, 2011).

In Southern Africa, in addition to point and non-point sources of pollution, climate change has also contributed to the long- term stresses on already compromised hydrological systems and water resources (Mwenge Kahinda et al., 2010). This extensive effect of climate change can impact not only freshwater resources, but in turn have detrimental effects on sustainable- and economic development, poverty reduction strategies, child mortality reduction programs, production and availability of food and the wellbeing of people and ecosystems (Rutashobya, 2008). As Southern Africa has become vulnerable to the impact of climate change, a few adaptive measures have been set in place in an attempt to alleviate the pressures placed on water sources. Moreover, countries around the world are exploring alternative water sources due to the negative impact of factors such as climate change and increasing population growth on current water resources.

Rainwater harvesting (RWH), which refers to the collection, concentration and storage of rainwater runoff, is an alternative technology that could assist in the provision of water directly to the household for drinking and hygiene purposes (Gould, 1999a). Millions of people are currently using RWH for drinking water purposes and an almost two-fold increase has been observed for both rural and urban users (WHO/UNICEF, 2012). One millimetre of rainwater collected per one square meter of collection surface equals one litre of water (FAO, 1985) and in South Africa and around the world, RWH has thus been earmarked as a short term intervention to provide water to especially dispersed settlement areas (DWA, 2009; DWA, 2012).

1.2 Domestic Rainwater Harvesting

Domestic rainwater harvesting (DRWH) involves the collection of water from rooftops, courtyards and low frequented streets. This independent water supply is then stored in water tanks, which can be built above or underground (Mwenge Kahinda et al., 2007), and primarily serves to supplement the main municipal water source. Depending on the requirements, storage tanks differ in size and shape and for the storage of smaller quantities of water, tanks are typically constructed from bricks, stabilized soil, rammed earth, plastic sheets and mortar jars (Helmreich and Horn, 2009). In order to store larger quantities of water, materials such as pottery, ferrocement or polyethylene can be utilised to construct the DRWH tanks.

While the initial capital costs associated with the installation of the tanks are moderate to high, most of the expenses are predominantly dependent on the on-site requirements, the size of the tank, the material used to construct the tank and whether the tank should be built above- or underground, while the "after installation" maintenance requires little or no expenditure (Gould, 1999b). In addition, provisional requirements for the use of a DRWH storage tanks include an adequate enclosure to minimize contamination from humans, animals and other environmental contaminants and a tight sealable cover, in order to prevent the growth of algae and the breeding of mosquitoes. Installing a DRWH tank thus allows for a readily available water source at the point of consumption. Furthermore, the World Health Organisation (WHO) has identified DRWH as an alternative improved water source as this technology could assist in the provision of water directly to the household for drinking and domestic purposes and could also provide water for small-scale home based productive activities, such as vegetable gardening, which could make a positive contribution towards food security for individuals from lower socio-economic groups (Gould and Nissen-Petersen, 1999; Mwenge Kahinda et al., 2010).

Hoff et al. (2010) described rainwater as "green water", that is, water that infiltrates the soil and is available immediately as rain falls and "blue water", which refers to water originating from a stream

or lake. Of the total precipitation one third of the rainwater will convert to blue water and two thirds constitute green water. However, as Awulachew et al. (2005) noted, unfavourable situations do arise. The variability in rainfall can be due to droughts and dry spells. Therefore an issue experienced with harvesting rainwater, is the lack of a continuous supply of rainfall even during unfavourable weather conditions. One solution to this problem is to increase the per capita storage for production and consumption purposes of individuals, or households, or communities. Nevertheless, in aiding the supply of fresh water to households, rainwater harvesting projects have continued to expand into various countries.

1.2.1 International rainwater harvesting projects

Globally, RWH is being recognised as an alternative water resource by a number of governmental organisations (Australian Government, 2011; Rowe, 2011; Lee et al., 2010a). Countries, including Australia, Canada, Denmark, Germany, India, Japan, New Zealand, Italy, Thailand and the United states have all investigated the potential benefits of rainwater harvesting (Despins et al., 2009; Evans et al., 2006; Uba and Aghogho, 2000). Favourable government policies have also directly promoted the increased implementation of harvested rainwater systems. For example, the United Kingdom has witnessed a 50% annual growth rate in the harvested rainwater market, and when France announced tax incentives for rainwater harvesting systems in 2008, the market grew by 50%. Rainwater harvesting has also allowed countries in the semi-arid areas of the world to provide local settlements with water, for example, it has previously been noted that 50% of Tanzania rely solely on rainwater as a water source (Mbilinyi et al., 2005). In addition, it has been reported that the government in Mozambique is funding large rainwater harvesting projects especially in the Gaza province, which represents the driest part of the country (UN, 2013).

There are, however, no international or national guidelines for routine rainwater analysis and monitoring and potential risks and hazards associated with rainwater harvesting need to be understood by likely users. In some instances local authorities have developed suitable manuals and guidelines for harvested rainwater use. For instance in Austin, Texas, the Texas Water Development Board in cooperation with other parties, published the third edition of The Texas Manual on Rainwater Harvesting in 2005. The purpose of the guide is to educate the public on rainwater harvesting and its applications. The authors describe the quality and treatment of harvested rainwater and the cost estimations and financial incentives involved in the implementation of DRWH systems (Krishna et al., 2005).

1.2.2 Rainwater harvesting projects in South Africa

The South African water sector is regulated and supported by the Department of Water Affairs (DWA), which is governed by two Acts, namely the National Water Act (1998) and the Water Services Act (1997), which together with national strategic objectives, oversees effective water use and management (DWA, 2013). South Africa's water resources typically comprise of 77% surface water, 9% groundwater, and 14% re-use of return flows (DWA, 2013). Unfortunately, there are many reports indicating the poor quality of surface water and it is predicted that there will be a decrease in the water recharge of underground water resources due to climate change, thus lowering the amount of water available in wells and boreholes (CSIR, 2009).

In South Africa, domestic rainwater harvesting has the potential to improve water availability amongst especially the rural communities, however, currently only 0.4% of the households in this country take advantage of utilising rainwater tanks (Statistics South Africa, 2010). In recent developments, the South African government, through the Department of Water and Affairs and Forestry (DWAF) and the Department of Science and Technology (DST), has made the distribution of tanks possible throughout five of the nine provinces, namely, the Eastern Cape, Limpopo, KwaZulu-Natal, Free State and the Western Cape. In these communities DRWH is an important source of drinking water, with approximately 14599 water tanks installed in rural areas in the Eastern Cape and 8275 water tanks installed in KwaZulu Natal (Mwenge Kahinda et al., 2010). South Africa, however, harbours large rural communities that are widely dispersed and peri-urban informal communities that are continuously expanding due to urbanisation. For these reasons decentralised water collection along with adequate water systems are in increasing demand and while rainwater harvesting projects have been successfully implemented in various regions of South Africa, there is little application in the major provinces (Mwenge Kahinda et al., 2007). This is a major concern as DRWH has the potential and has been ear-marked to act as an alternative technology to decentralise water collection and treatment systems in especially rural areas.

While DRWH allows for domestic activities such as vegetable gardening which aids in food security (Awulachew et al., 2005), information regarding the presence and risk of chemical and microbial pollutants is limited and for this reason, DRWH has not been widely used in South Africa or internationally (Ahmed et al., 2011a). As the potential public health risks associated with untreated rainwater have not been identified, no legislation regarding the use of rainwater in South Africa has been stipulated (Mwenge Kahinda et al., 2007). This is of major concern as it has previously been noted that in order to use harvested rainwater as part of a solution to the water crisis, rainwater needs to be qualitatively and quantitatively assessed (Eruola et al., 2013). The

quality of the rainwater however, depends on several factors such as the weather conditions, proximity of the pollution source, the maintenance of the water tanks, the type of catchment area and the topography of the rainwater tank (Mwenge Kahinda et al., 2007).

1.3 Protective Measures in Preserving Rainwater Quality

There are a few factors to consider when selecting a storage tank as well as the appropriate design of the harvesting and storage system. These factors include the desired area, together with the topography, the weather conditions, the proximity to pollution sources, the type of catchment area, the type of water tank and the handling and management of the water harvesting system (Gould, 1999b; Zhu et al., 2004; Sazakli et al., 2007).

1.3.1 Appropriate system design

As suggested by Mwenge Kahinda et al. (2007) the design of the collection process should be geared towards lowering the amount of pollution and even though the design and the management of the tank system can minimize the pollution load, it is impractical to assume that harvested rainwater will reach drinking water standards without the appropriate treatment. Numerous entry points for chemical and microbial contaminants into the DRWH tank have been described, which range from dust that blows from the rooftop into the tank, to other vectors such as mosquitoes that gain direct access.

One of the major concerns of utilising harvested rainwater as a sole water supply, is the lack of a continuous supply of water through the dry spells and low rainfall periods (Mwenge Kahinda et al., 2007). There is, however, a limit on the amount of available rainwater that can be harvested per day, per week and per year, for any given size and location (DTU, 2001). To ensure the availability of water, a few factors should be taken into consideration: the availability of water, which includes the rainfall patterns, the catchment area and the presence of an alternative water supply; the amount of water required, which varies from one rainfall area to the next; technical constraints, such as whether the tank should be installed above- or underground; whether or not the catchment area is built from thatch, which has a lower runoff coefficient than corrugated iron sheets; the size of the area where the tank is to be installed, and lastly; socio-economic constraints, which is an important factor to consider since most South Africans cannot afford the cost of a storage tank as approximately 67% of rural households are below the poverty line (Statistics South Africa, 2003).

1.3.2 Operation and maintenance

Potential sources of contamination associated with harvested rainwater include, amongst others, dust, leaves and bark from trees, insects, and animal droppings from for example rats and birds that fall into the catchment area. Therefore as part of routine maintenance authors such as Sazakli et al. (2007) advised the regular cleaning of the catchment area and the interior of the rainwater tank system. However as these tasks are not always practically possible, the installation of first flush divers which act in eliminating the first few millimetres of rain have been advised.

1.3.3 First flush diverters

For the improvement of the quality of harvested rainwater, it has also been advised that the catchment surfaces be cleaned regularly and diverting devices be installed. In a country such as South Africa where rainfall is sporadically distributed, the first few millilitres of rain should be diverted or flushed after dry spells (Martinson and Thomas, 2005). Mwenge Kahinda et al. (2007) advised that consistent cleaning of the catchment area and the installation of a diverting device will improve the water quality. Certain studies however suggest that first flush diverters do not minimise the microbial contamination found in harvested rainwater. Ror example, Gikas and Tsihrintzis (2012) noted that the installation of a first flush rainwater diverter improved the physicochemical quality of the harvested rainwater, but did not improve the concentration of microbial pollutants.

1.4 Chemical Pollutants Associated with Rainwater Harvesting

There are conflicting studies regarding the pollution and safety of rainwater and this could be as a result of many dissimilar variables present within each system (Ahmed et al., 2011a). Due to the acidic nature of rainwater, roofing can affect the chemical quality of harvested water as materials may leach into the runoff (King and Bedient, 1982). Moreover, roof temperatures are much higher compared to the temperatures of other surfaces. Therefore pollutants such as heavy metals and organic substances may also result due to leaching from the particular catchment material and compounds that accumulate on the roof top surfaces (Chang et al., 2004). Studies have, however, shown that roofs constructed from tiles, slates, and aluminium sheets are the most effective in harvesting low or non-polluted water. Roofs made from zinc or copper, along with roofs painted with metallic paints, are less desirable, as these contain elevated concentrations of heavy metals compared to catchment areas constructed with tiles, slates, and aluminium sheets (Gould, 1991).

As previously noted, the increased temperatures of roofing materials may accelerate chemical reactions and organic decomposition in the catchment area. Chemical contaminants (aerosols and

gases) may also occur as a result of airborne pollutants. Particles, microorganisms, heavy metals and other organic substances are some of the major pollutants found in the atmosphere that can potentially affect harvested rainwater. The washout process or the rainout process may also possibly be responsible for the chemical groups that have been discovered in precipitation. Material may be transported to cloud droplets, before rain droplets make their descent (rainout), and during the washout process, material is transferred to falling rain droplets (Burch et al., 1996). Some studies showed that concentrations of ions and heavy metals decreased when the rainfall increased (Alastuey et al., 2001; Hou et al., 2005; Huston et al., 2009). Huston et al. (2009) showed how location may have an effect on the continual distribution of deposited contaminants. For example, urban areas are generally detached from the pressures of atmospheric and industrial pollution, but high traffic, which is characteristic of urban areas, pollutes the atmosphere with an assortment of particles, heavy metals and organic air pollutants.

Reports have shown that chemical pollutants present in harvested rainwater are generally within the WHO drinking water quality standards (Haebler and Waller, 1987; Scott and Waller, 1987; Michaelides, 1986; 1989). However, a study conducted by Wirojanagud et al. (1989) showed slightly elevated concentrations of magnesium and zinc, which exceeded the WHO (1993) guidelines in collected rainwater samples. These elements are not usually associated with severe health issues, however, the presence of elevated levels of lead [exceeding the New Zealand Drinking Water Standards (NZDWS) (Ministry of Health, 1995)], as was observed in harvested rainwater in New Zealand, was a cause for concern (Simmons et al., 2001). As determined by Gould (1999b) several sources of lead poisoning exist in a rainwater harvesting system, including the use of lead flashing, lead headed nails, lead based painters/ primers or industrial or traffic activities, which results in atmospheric pollution. In a study completed in Greece by Gikas and Tsihrintzis (2012), six rainwater harvesting systems were used to determine, amongst other contaminants, major anions (nitrite and nitrate, fluoride, chloride) and cations (ammonium, sodium, potassium, calcium and magnesium). All major chemical parameters were within the 98/93/EU directive for drinking water, with the exception of ammonium. As noted in the study, ammonium and phosphorus normally originate from natural sources, such as bird faeces and lichens that contaminate the roof runoff, especially in areas with low industrial activity. The results of studies that have performed extensive chemical analysis on harvested rainwater are summarised in Table 1.1. For example, Morrow et al. (2010) sampled 5 out of 10 rainwater tanks in a study, which examined various potential chemical pollutants. Various sampling points such as the catchment area (before entering the tank) and the cold water taps (after entering the tank) were also examined.

Table 1.1 Extensive chemical analysis of harvested rainwater performed by previous studies

Parameter	East Coast, Australia [a]	Bermuda[b]	Gangneung, South Korea[c*]	National study, Australia[d]	Brisbane, Australia[e]
Magnesium	387	1500	1200	700	500
Copper	5.11	1.9	85	-	21.00
Phosphorus	18	-	-	-	-
Lead	228	0.47	27	-	5.40
Sodium	3148	1300	3200	3500	2800
Lithium	-	-	-	-	0.55
Potassium	50	1200	3100	600	900
Manganese	16	BDL	115	10.2	8.70
Zinc	16	23	160	1790	770
Iron	0.12	17	-	-	68
Rubidium	0.12	-	-	-	-
Nickel	BDL	BDL	-	2.0	1.30
Silver	BDL	BDL	-	-	-
Selenium	0.19	0.62	-	BDL	-
Cobalt	0.05	ND	-	0.7	0.17
Arsenic	0.05	BDL	3	1.0	0.25
Vanadium	1.56	2.6	-	-	0.32
Barium	0.37	5.0	-	6.4	12.0
Chromium	0.27	0.98	4.5	9.8	0.53
Bismuth	BDL	BDL	-	-	-
Cadmium	0.01	BDL	1.5	0.9	-
Strontium	638	160	-	15.2	30.00
Tin	BDL	BDL	-	BDL	0.51
Caesium	BDL	-	-	-	-
Titanium	BDL	BDL	-	-	-
Uranium	BDL	BDL	-	-	-
Molybdenum	BDL	0.20	-	BDL	-
Calcium	-	15	6.4	3.7	2.4
Aluminium	-	130	225	41.6	314
Boron	-	BDL	-	-	-
Beryllium	-	ND	-	BDL	-
Antimony	-	0.86	-	BDL	0.15
Thallium	-	BDL	-	-	-
Mercury	-	BDL	-	0.4	-
Bicarbonate HCO_3^-	-	-	-	20900	-
Chloride Cl^-	-	3000	7500	-	3900
Nitrate NO_3^-	-	5000	6800	1200	1600
Nitrite NO_2^-				6	600
Sulphate SO_4^{2-}	-	9700	4100	3200	1600
Ammonia NH_3^+	-	-	90	74	-
Phosphate PO_4^{3-}	-	-	20	-	100

BDL- Below Detection Level
All concentrations were measured in µg/L
[a] Morrow et al. (2010)
[b] Peters et al. (2008)
[c*] Lee et al. (2010b) (Median values)
[d] Chapman et al. (2008)
[e] Huston et al. (2012)

It was noted that the tanks were relatively new (5 – 10 years old) and the catchment area, as well as the down pipes and plastic, was constructed from Colorbond™. For this reason the values indicated in Table 1.1, for the Morrow et al. (2010) study, represent samples that flowed from the catchment

area, into a tank and out through a tank tap. It was noted that only iron concentrations were significantly higher in roof run off samples compared to tank water samples. Differences in the elemental composition of the collected rainwater differed between sites and collection points. The piping material used in the construction of the rainwater harvesting systems also contributed significantly to the total elemental load rather than the contaminants found in the atmosphere. In another study performed in Australia, Huston et al. (2012) determined the proportion of contaminants originating from different sources by making use of the Positive Matrix Factorisation model, EPA PMF 3.0 (USEPA, 2008). It was concluded that 65% of the contaminants could be attributed to atmosphere contamination and plumbing, building material used, galvanizing, roofing and steel, while lead flashing/paint contributed to 35% of the total chemical contamination. Of the rainwater tanks sampled, 15% exceeded the Australian Drinking Water guidelines (NHMRC, 2004).

Elevated levels of metals may also be released into the environment through both natural and human activities. Examples of anthropogenic sources include fossil fuel combustion, vehicle exhausts, agricultural chemicals and liquid and solid waste from humans (Fishbein, 1982). Fishbein (1982) acknowledged that toxic trace metals and metalloids that are able to leach out of the environment represent harmful forms of pollution. The author suggested that arsenic, beryllium, cadmium, chromium, lead, mercury, nickel, and selenium amongst others are some of the most important chemical pollutants. The Department of Water Affairs and Forestry stated in the South African Water Quality Guidelines (1996) that even at very low concentrations, cadmium, chromium, lead, mercury and vanadium can exhibit either acute or irreversible effects on human health.

1.5 Microbial Pollutants Associated with Rainwater Harvesting

Numerous countries throughout the world use rainwater harvesting as an important source of water for domestic purposes, especially in rural areas. Traditionally, however, the method of catchment of harvesting rainwater from rooftops, courtyards or low frequented streets, could allow pathogens from animal droppings and other organic debris to be flushed into the tanks via the gutters and inlet tank system. Moreover, a link between the rainwater harvesting system and bird and animal debris was then established in a study performed in Southeast Queensland, Australia, where identical biochemical phenotype profiles of *Escherichia coli* strains were isolated from RWH tanks and from bird and possum faeces found on the roof surface. These results suggested that the faeces could have been the source of *E. coli* contamination in the RWH tanks (Ahmed et al., 2012b).

Although no study has clarified a correlation between the amount of rainfall and the contaminants present in the harvested rainwater, many international studies, which investigate the microbial

quality of rainwater, are conducted during the rainy seasons and samples are collected one to four days after a rain event (Ahmed et al., 2008; 2011b; 2012a; b; Handia, 2005). While Dillaha and Zolan (1985) and Sazakli et al. (2007) conducted their studies over a 12-month period, seasonal variation was only considered in the Sazakli et al. (2007) study, where the highest microbial counts were recorded during the autumn sampling period. However, as guidelines for rainwater quality have not been stipulated, it is common practice to use guidelines of drinking water to monitor the chemical and microbial quality of rainwater.

1.5.1 Guidelines for monitoring the microbial quality of (rain)water

In South Africa, guidelines such as the South African Water Quality Guidelines, volume 1, Domestic Use, published by the South African Department of Water Affairs and Forestry (DWAF) in 1996, and the South African National Standard (SANS) 241 for Drinking Water Quality, published by the South African Bureau of Standards, are used as guidelines in monitoring the quality of drinking water (SANS, 2005).

It would however, be impractical to assess rainwater sources for all possible pathogen contaminants and for this reason many studies were aimed at detecting indicator organisms (Table 1.2) (De Kwaadsteniet et al., 2013). To allow for a reliable indication of the potential risks of infection, a combination of indicators, such as total coliforms (TC), faecal coliform (FC), *Escherichia coli* (*E. coli*) and enterococci (ENT), are routinely tested for. Coliforms are a large group of Gram negative, non-spore forming, rod shaped bacteria belonging to the family, *Enterobacteriaceae* (Willey et al., 2008) and TC bacteria serve as indicators of the general hygienic quality of the water. In addition, the enumeration of FC bacteria serves as an indicator of the level of faecal pollution in the water source as these microorganisms originate from a faecal source and can grow at higher temperatures than total coliforms.

Escherichia coli is the only member of the coliforms and thermotolerant coliforms that is found exclusively in faeces and does not noticeably multiply in the environment (Edberg et al., 2000). It is thus included as a specific indicator organism of faecal pollution from warm-blooded animals and only the enumeration of *E. coli* indicates a true level of faecal contamination (Environmental Agency, 2002). Enterococci or faecal streptococci survive longer in water compared to coliform bacteria but may also be used as specific indicators of faecal pollution. Species consist of *Enterococcus faecalis*, *E. faecium*, *E. durans*, *E. hirae*, *E. cecorum*, *E. columbae*, *E. avium* and *E. gallinarum* together with *Streptococcus bovis* and *S. equinus* (ISO, 1998; Ashbolt et al., 2001; WHO, 2003).

Heterotrophic bacterial plate counts (HPC) serve as an indirect indicator of water quality and they are usually included to determine the number of culturable bacterial organisms in a particular water source (Lye et al., 2002). Some studies suggest that the enumeration of the HPC allows for the monitoring of the disinfection efficiency in a system, which implies that if the plate counts are above a particular standard, measures can be implemented to lower the level of microbial contamination. However, as the term heterotrophic plate count refers to the enumeration of a fraction of the bacterial species that are able to utilise organic nutrients for growth, these numbers may include non-pathogenic species, primary and secondary bacterial pathogens along with coliforms (Allen et al., 2004).

1.5.1.1 Viral indicators

One of the short comings of utilising indicators is that numbers vary in water environments as individuals do not always excrete pathogens, and these numbers are greatly affected by scenarios such as disease outbreaks (DWAF, 1996). In addition, naturally occurring water body processes such as sedimentation, absorption and coagulation, may render the faecal indicator undetectable as some of these processes may provide protection for bacteria from inactivation through treatments. For this reason, a number of studies have shown that faecal indicators do not necessarily correspond to bacterial pathogen numbers present and the detection of certain bacteriophages (viruses that infect bacteria) to assess water quality is growing increasingly popular. Amongst the bacteriophages, coliphages are divided into two large groups, somatic coliphages and male- specific coliphages (Grabow, 2004). Somatic coliphages (those that are able to infect and replicate in *E. coli*) are easily detectable with simple, economic and rapid techniques, and studies have shown that they occur in large numbers in sewage and polluted water environments (Grabow et al., 1993). However, these phages are able to replicate in water environments. For this reason male-specific (F-RNA) coliphages which are commonly found in sewage water and cannot replicate in water environments are highly specific indicators of faecal pollution (Grabow et al., 1980). These F-RNA coliphages would thus offer a superior representation of human viruses when compared to somatic coliphages. As they infect and replicate in *E. coli*, they represent highly specific indicators for the presence of faecal pollution by warm-blooded animals, including humans (Grabow, 2001). However, detection methods for F-RNA phages are known to be more complex than those for the detection of somatic coliphages. In natural waters contaminated with sewage, coliphages can also be detected in high numbers and somatic coliphage counts range from 10^3 plaque forming units (pfu)/mL to 10^4 pfu/mL in sewage (Grabow et al., 1993; Tartera et al., 1989). To date, no reports have however, determined coliphage PFUs or determined the presence of human enteric viruses in harvested rainwater.

1.5.2 Reports on microbial indicators in rainwater

Conflicting reports on the quality of the rainwater source have been published (Table 1.2). In many investigations the authors conclude that based on the significantly high TC, FC, ENT and *E. coli* counts recorded in the samples analysed, rainwater is not suitable for drinking purposes, with limited application for domestic use (Yaziz et al., 1989; Sazakli et al., 2007; Zhu et al., 2004). In contrast studies conducted by Dillaha and Zolan (1985) in Micronesia (203 catchments on 10 islands) and by Thomas and Green (1993) in Australia, described harvested rainwater as generally acceptable for drinking and household use.

New Zealand relies on roof-collected rainwater for potable domestic supply, especially in rural households where this collected rainwater can be seen as the only available domestic water source (Simmons et al., 2001). A study completed by Simmons and co-workers (2001) in Auckland, New Zealand, however, demonstrated that the rainwater is not always safe and based on the level of indicator organisms present, it cannot always be considered potable. This study involved investigating the level of microbiological contamination in 125 samples from four rural districts. Heterotrophic plate counts (HPC), TC, FC and ENT were analysed as indicator organisms in 56% of the water supplies exceeded the criteria stipulated in the New Zealand Drinking Water Standards (NZDWS) of < 1 FC/100 mL. A significant positive correlation between *Aeromonas* spp. and bacterial indicator organisms was also found.

Rainwater harvesting programmes have also been successfully initiated in the Mediterranean region and in particular the Greek islands, due to increasing water demand and the effect of climate change on water availability (Global Water Partnerships, 2011). A study conducted by Sazakli et al. (2007) on the quality of harvested rainwater used for domestic and drinking purposes in the northern area of Kefalonia Island in South West Greece and the factors affecting it, were assessed. In their 3-year surveillance the presence of coliforms, *E. coli* and enterococci in harvested rainwater samples were found to be 80.3%, 40.9% and 28.8%, respectively. In addition, the authors concluded that the rainwater sources lacked fluoride and suggested that the supplementation of this element was required.

Rainwater harvesting and the quality of the harvested water has also been extensively studied in Australia. It is reported that 10% of the Australian people use harvested rainwater as their drinking source and a further 5% have been known to use the harvested rainwater for showering, toilet flushing, and clothes laundering (ABS, 2007). To encourage the use of harvested rainwater, the Queensland State Government, Australia, initiated the “Home Water Wise Rebate Scheme” in 2006, as part of a subsidy scheme for residents that were able to utilise rainwater for non-potable

domestic purposes. The aim of the scheme was to decrease the residents' reliance on the municipal scheme water (Ahmed et al., 2011a). However, reports on the quality of harvested rainwater in Australia have all shown that the faecal indicators are not within the Australian Drinking Water guidelines as indicated in Table 1.2.

Table 1.2 Percentage of samples that tested positive (> 1 CFU/100 mL) for total bacteria and faecal indicators (number of samples selected indicated in brackets) (adapted from Ahmed et al., 2011a)

Country	Total Bacteria	Total Coliforms	Faecal Coliforms	*E. coli*	Enterococci	Reference
Australia	NR	52 (100)	38 (100)	NR	NR	Verrinder and Keleher (2001)
Australia	NR	90 (49)	NR	33 (49)	73 (49)	Spinks et al. (2006)
Australia	NR	NR	NR	63 (27)	78 (27)	Ahmed et al. (2008)
Australia	NR	NR	NR	58 (100)	83 (100)	Ahmed et al. (2010)
Australia	100 (67)	91 (46)	78 (41)	57 (67)	82 (67)	CRC for Water Quality and Treatment (2006)
Australia	NR	NR	83 (6)	NR	NR	Thomas and Green (1993)
Australia	100 (77)	63 (81)	63 (81)	NR	NR	Evans et al. (2006)
Canada	NR	31 (360)	14 (360)	NR	NR	Despins et al. (2009)
Greece	NR	80 (156)	NR	41 (156)	29 (156)	Sazakli et al. (2007)
Denmark	100 (14)	NR	NR	79 (14)	NR	Albrechtsen (2002)
Italy	ND	152 (NR)	ND	328 (NR)	ND	Naddeo et al. (2013)
Micronesia	NR	43 (155)	70 (176)	NR	NR	Dillaha and Zolan (1985)
New Zealand	NR	NR	56 (125)	NR	NR	Simmons et al. (2001)
Nigeria	100 (6)	100 (6)	ND	NR	ND	Uba and Aghogho (2000)
South Korea	NR	92 (90)	NR	72 (90)	NR	Lee et al. (2010b)
Thailand	NR	NR	NR	40 (86)	NR	Pinfold et al. (1993)
USA	100 (30)	93 (30)	NR	3 (30)	NR	Lye (1987)
U.S. Virgin Islands	86 (45)	57 (45)	36 (45)	NR	NR	Crabtree et al. (1996)
U.S. Virgin Islands	NR	NR	59 (17)	NR	NR	Ruskin and Krishna (1990)
Hawaii- USA	NR	NR	89 (9)	NR	NR	Fujioka et al. (1991)
Zambia	NR	100 (5)	100 (5)	NR	NR	Handia (2005)

*NR= Not Reported
*ND= Not Determined

In South Africa, studies focus predominantly on the optimisation of the tank system and it is recommended that factors such as water availability and requirements, and technical constraints such as the limitation of space and the socio-economic pressure, need to be taken into account (Nevondo and Cloete, 1999). Nevondo and Cloete (1999) conducted a study on the quality of rainwater in Hammanskraal, which is situated 55 km north of Pretoria, Gauteng. They found the HPC to be between 1.0 x 10^1 and 1.63 x 10^4 CFU/ mL, with an average count of 3.27 x 10^3 CFU/ mL, while the TC counts were between 4.7 x 10^2 and 1.0 x 10^3 CFU/ mL, with the FC count ranging between 9.0 x 10^1 and 2.6 x 10^2 CFU/ mL. From the HPC, TC and FC counts the general quality of the rainwater source, was deemed unacceptable. Numerous studies have, however, also focussed on detecting common bacterial pathogens in harvested rainwater.

1.5.3 Bacterial pathogens associated with rainwater

Animals that have access to the catchment areas can be responsible for the presence of undesired bacteria, viruses and protozoa in the rainwater source as pathogens that occur in the faeces of birds, insects, mammals and reptiles can contaminate the water collected from roof surfaces and streets. For this reason bacterial pathogens often associated with water sources and animal faeces such as *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp. and *Aeromonas* spp., are often tested for in surveys assessing the quality of rainwater (Table 1.3). Simmons et al. (2001) monitored harvested rainwater samples for the presence of *Salmonella* spp., *Legionella* spp., *Campylobacter* spp. and *Aeromonas* spp. and the protozoan species, *Cryptosporidium* and *Giardia*. *Salmonella* spp. were detected in 0.9% of the water samples, while *Legionella* spp. and *Campylobacter* spp. were not detected. The presence of protozoan species were only determined in samples that contained elevated levels of FC or ENT, with *Cryptosporidium* oocysts detected in 4% of the selected 50 samples, while *Giardia* was not detected.

Pseudomonas aeruginosa is commonly found in faeces, soil, and certain food and water sources. It cannot be used as a faecal indicator as it is not universally present in faeces and sewage. In addition, suitable surfaces of organic material that come into contact with water and the enrichment of water, will allow for *P. aeruginosa* to multiply (NHMRC and NRMMC, 2011). In Australia, the presence of *P. aeruginosa* indicates the general cleanliness of water (NHMRC and NRMMC, 2011). This organism may colonise damaged tissues of its host, for instance burn wounds, making it a classical opportunistic pathogen. Colonisation of *P. aeruginosa* in wounds, could then lead to the creation of critical lesions or septicaemia (NHMRC and NRMMC, 2011).

Legionella is found in natural freshwater sources and soils, and in man-made water systems such as hot water and cooling systems. *Legionella pneumophila* serogroup 1 is one of 26 species that is

repeatedly and predominantly associated with human disease. The species is thought to infect humans by inhalation, rendering its presence in drinking water irrelevant. But its growth is amplified under certain conditions, usually by thermal enhancement, for example hot water systems can form aerosols in the nozzle heads of showers, which allow these organisms to proliferate. There are two types of diseases associated with *Legionella* infections, legionellosis, also known as Legionnaires' disease and Pontiac fever (NHMRC and NRMMC, 2011).

Table 1.3 Bacterial pathogens associated with DRWH (adapted from Ahmed et al., 2011a)

Country	Positive Percentage of Samples	Pathogenic Bacteria Detected	Reference
New Zealand	20	*Aeromonas* spp.	Simmons et al. (2001)
	0.9	*Salmonella* spp.	
	4	*Cryptosporidium* spp.	
Nigeria	83	*Pseudomonas* spp.	Uba and Aghogho (2000)
	67	*Salmonella* spp.	
	67	*Shigella* spp.	
	67	*Vibrio* spp.	
U.S Virgin Islands	45	*Cryptosporidium* spp.	Crabtree et al. (1996)
	23	*Giardia* spp.	
U.S Virgin Islands	80	*Legionella* spp.	Broadhead et al. (1988)
Australia	15	*Aeromonas* spp.	Ahmed et al. (2008)
	26	*Legionella* spp.	
	45	*Campylobacter* spp.	
	11	*Salmonella* spp.	
	19	*Giardia* spp.	
Australia	7	*Aeromonas* spp.	Ahmed et al. (2010)
	8	*Legionella* spp.	
	20	*Campylobacter* spp.	
	17	*Salmonella* spp.	
	15	*Giardia* spp.	
Australia	32	*Aeromonas* spp.	CRC for Water Quality and
	15	*Legionella* spp.	Treatment (2006)
	1.5	*Campylobacter* spp.	
	3	*Salmonella* spp.	
Denmark	14	*Aeromonas* spp.	Albrechtsen (2002)
	7	*Pseudomonas* spp.	
	71	*Legionella* spp.	
	12	*Campylobacter* spp.	
	7	*Mycobacterium* spp.	
	35	*Cryptosporidium* spp.	
New Zealand	37	*Campylobacter* spp.	Savill et al. (2001)

Shigella, which is genetically closely related to *E. coli*, are highly pathogenic towards humans and cause bacillary dysentery, which is an infectious disease of the intestinal tract. *Shigella* infection is not usually water-borne, but major outbreaks have been reported as a result of transmission of this organism by water. The isolation of *Shigella* spp. from water serves as an indication of recent human faecal contamination. There is no enrichment or selective media for only *Shigella* spp.,

which could clarify why they are very rarely detected, even though they could be present in various water bodies (NHMRC and NRMMC, 2011).

Aeromonas spp. are found in freshwater, soil and food mainly meat, fish and milk. They are classified in the family *Vibrionaceae*. The genus is subdivided into two groups, the psychrophilic, non-motile group consisting of *Aeromonas salmonicida* (a fish pathogen) and a mesophilic, motile group consisting of *A. hydrophila, Aeromonas sobria* and *Aeromonas caviae*. The mesophilic group also causes infections in cold-blooded animals, and has been implicated in infections in immune-compromised patients. They are able to cause septicaemia and have been linked with gastroenteritis in children (Gracey et al., 1982).

Thermophilic (growing at 42°C) *Campylobacter* spp. can cause gastrointestinal illness. Many species are pathogenic including *Campylobacter jejuni, Campylobacter coli* and *Campylobacter fetus,* while non-pathogenic species include *Campylobacter sputorum* and *Campylobacter concisus* (Penner, 1988). Wild birds and poultry are the major carriers of *Campylobacter*. However, other domestic animals, including pigs, cattle, dogs and cats, can also harbour the thermophilic *Campylobacter* bacteria. Depending on rainfall, temperature, and the presence of birds, *Campylobacter* spp. can be found in surface waters as they survive well at low temperatures. A number of outbreaks of campylobacteriosis have been reported in the last decade due to the presence of *Campylobacter*, but only two of them were as a result of a contaminated non-chlorinated water source (NHMRC and NRMMC, 2011).

The presence of *Salmonella* in water bodies is as a result of faecal contamination from animals and inadequately treated waste discharges. *Salmonella* is broadly dispersed in the environment and gain access to water systems in this way. These are also the main causes of salmonellosis outbreaks. *Salmonella enterica* serovar Typhi is a human pathogen, whereas other species use animals as reservoirs, which could aid in the transferal of the pathogen to humans (Lloyd, 1983).

1.5.4 General protozoan pathogens associated with harvested rainwater

Cryptosporidium is an obligate parasite that is capable of reproducing sexually and asexually. The presence of thick-walled oocysts in faeces are accountable for the transmission of this protozoan species. Oocysts can survive for weeks in fresh water under cool conditions (King and Monis, 2007) and *Cryptosporidium* is thus regarded as one of the most significant water-borne human pathogens in developed countries. North America and Britain have reported 30 outbreaks, which have all been associated with drinking water. According to Mackenzie et al. (1994) the largest number of people that were affected by this protozoan was estimated to be around 403 000. The

methods for testing for the presence of this human pathogen remain demanding and relatively expensive, but have become increasingly reliable. *Cryptosporidium hominis* and *Cryptosporidium parvum* have been acknowledged as the major causes of disease, known as cryptosporidiosis, in humans. However, *C. hominis* affects humans, while the *C. parvum* strains that infect humans can also occur in cattle and sheep.

Giardia has been considered a serious water-borne, human pathogen since the 1960s. However, the tests for detecting this infectious species in water are limited. *Giardias*' lifecycle consists of two phases. In the intestine, it exists in its flagellated phase which is able to multiply. Whereas in faeces it occurs in elevated numbers and appears as thick-walled cysts that shed sporadically. Wallis et al. (1996) reported 240 cysts per litre in surface water, and in Australia, sewage typically presented much larger prominent numbers of *Giardia* than *Cryptosporidium*. Similar to *Cryptosporidium*, *Giardia* has robust cysts that are able to survive in water for weeks and outbreaks of human infections (giardiasis) occur when untreated water is ingested. *Giardia* species are also found in a wide range of hosts from birds (*G. psittaci)*, other mammals (*G. muris)*, to amphibians (*G. agilis)*, with *Giardia lamblia* (syn. *Giardia intestinalis*, *Giardia duodenalis*) considered the primary pathogen of humans and other mammals.

1.6 Incidences of Disease Associated with Harvested Rainwater

Gastroenteritis associated with the consumption of harvested rainwater has been recorded in many cases in literature, with several authors demonstrating the elevated health threat in this water source (Koplan et al., 1978; Murrell and Stewart, 1983; Schlech et al., 1985; Brodribb et al., 1995; Simmons and Smith, 1997; Merritt et al., 1999; Simmons et al., 2001; Lye, 2002; Ahmed et al., 2008; Simmons et al., 2008; Franklin et al., 2009). Adopted from Ahmed et al. (2010), Table 1.4 shows the various pathogenic bacteria that have been linked to reported cases of disease associated with the consumption of harvested rainwater.

Birds nesting in the catchment areas of rainwater collection systems in New Zealand were hypothesised to be causing campylobacteriosis in certain cases where the water was consumed (Eberhart-Phillips et al., 1997). In another incident microbiological studies were undertaken after a group of tourists were reported to have contracted Legionnaire's disease whilst visiting the U.S. Virgin Islands. The exact mode of transmission was not established, but the hotel acquired its potable water from a DRWH system, which seemed to be the most likely cause as an identical serogroup of *Legionella pneumophila* was isolated from the patients, the stored harvested rainwater and the hot and cold water outlets. The hotel then resorted to chlorinating the water and no further outbreaks were reported (Schlech et al., 1985). *Salmonella mississippi* was also implicated as the

cause of infections in Tasmania, Australia, after individuals had consumed water from contaminated harvested rainwater (Ashbolt and Kirk, 2006).

Table 1.4 Reported cases of disease associated with the consumption of harvested rainwater

Country	Pathogenic Microorganism	Contracted Disease	Individuals Affected	Reference
Australia	*C. botulinum*	Not specified	3	Murrell and Stewart (1983)
Australia	*Campylobacter fetus*	Diarrhoea, vomiting	1	Brodribb et al. (1995)
Australia	*Campylobacter* spp.	Diarrhoea, abdominal pain	23	Merrit et al. (1999)
Australia	*Salmonella typhimurium* phage 1	Diarrhoea, abdominal pain, nausea	27	Franklin et al. (2009)
New Zealand	*S. typhimurium* phage 1	Diarrhoea	2	Simmons and Smith (1997)
New Zealand	*L. pneumophila*	Legionnaires' disease	1	Simmons et al. (2008)
U.S.Virgin Islands	*L. pneumophila* serogroup 1	Legionnaires' disease	27	Schlech et al. (1985)
West Indies	*Salmonella arechevalata*	Diarrhoea, headache, fever, vomiting	48	Koplan et al. (1978)

Ahmed et al. (2010) suggested that it may be likely that the incidences of gastrointestinal outbreaks associated with RWH tanks are not always reported as not every individual seeks medical advice or attention. In addition most faecal specimens that are collected are not always extensively analysed in hospitals. They also suggested that most communities considered the quality of harvested rainwater equal to potable water and would rather blame other sources for infection before implicating DRWH as the potential source of a disease. It was also reported that only between 8 and 11% of *Campylobacter*- and *Salmonella*-linked food-borne gastroenteritis cases are reported in Australia (Hall et al., 2006) and only 10 to 33% of gastroenteritis cases associated with water are reported in America (Frost et al., 1996). In South Africa, water-related diseases take on a new dimension as South Africa has one of the highest HIV/AIDS prevalence rates in the world and it is estimated that 5.5 million people are infected (UNAIDS, 2006). Unfortunately diseases associated with the ingestion of rainwater have not yet been reported and this is probably due to the limited number of surveys that have been conducted (Nevondo and Cloete, 1999; Mwenge Kahinda et al., 2007). However, as mentioned previously, of the health implications associated with utilizing domestic rainwater harvesting, the storage of water which may allow for insect vector breeding should be addressed as a priority (Vasudevan et al., 2000), as water related diseases transmitted by vectors which breed in water include, dengue fever, filarialsis, malaria, onchocerciasis, trypanosomiasis and yellow fever (Eisenberg et al., 2001). Detailed monitoring studies, however, need to be undertaken to accurately determine the microbial quality of the rainwater. Where

applicable, measures such as the implementation of filtering systems and solar panels can then be implemented to improve the quality of the water to within drinking water standards.

1.7 Rainwater Treatment Systems

The collection and transportation of water to households from a particular water source is not only a cumbersome task but the quality of the available water should not be compromised during the transportation or storage process (Nieuwoudt and Mathews, 2005). As noted previously the need for water treatment systems that are effective in the developing world is undeniable (Burch et al., 1998). Besides the inconvenience of not having a water source in close proximity to households, of concern, is the fact that approximately 5000 children under the age of five die every year because of a diarrhoeal disease, with an estimated 1800 of these deaths related to water, sanitation and hygiene. Associated illnesses, is a major concern as not only do episodes of diarrhoea leave the victim debilitated and malnourished, but it also increases their vulnerability to other diseases (UNICEF, 2012; World Gastroenterology Organisation, 2012).

The general issues associated with water treatments are highlighted in Burch et al. (1998) where the authors divide the technical and social variables into twelve categories. A point of use treatment system that will potentially provide safe and adequate water for drinking is a priority, especially in situations where there is a lack of treated water supplies, or where the treated water supplies have been compromised. Mwabi et al. (2011) stated that the best technology to be used will depend on a particular situation. For this they noted that factors including the quality of the water source, the availability of the required materials and equipment, the time frame in which the system is to be used, the customs of the communities, the preferences and the education levels of the local population and the availability of personal to provide the necessary guidance, training and monitoring for each system, will have to be taken into consideration during the implementation of a treatment system. Various disinfectants such as chlorine and iodine and treatments including, filtration, distillation, reverse osmosis and solar disinfection have all been shown to decrease microbial contamination and improve the chemical quality of water (Sobsey, 2002; Murcott, 2006; Elliot et al., 2008).

The effectiveness of a treatment system will however, greatly depend on the turbidity and the types of pathogens present in the water source. Turbidity is the measure of the concentration of solid particles suspended in water; this is commonly determined by light scattering and expressed as nephelometric turbidity units (NTU). These small particles represent organic matter, for example faecal matter or colloids which are micron-sized clay particles. These particles are known to decrease the effectiveness of ultraviolet (UV) radiation and the particles often serve as shields for

microorganisms against UV and chemical disinfectants. Also, high turbidity levels can cause filters to become clogged rapidly, and this in turn increases the maintenance required for filters. The turbidity of clear water, such as water originating from a well is generally low (< 10 NTU), while the turbidity in dirty rivers and lakes can range from 10 to 2000 NTU (Burch and Thomas, 1998).

The types of pathogens present will also affect the choice of water treatment system, for example, viruses are difficult to remove by filtration, as they are very small. Protozoa are known to form cysts when under stress and worms lay eggs. Cysts and worm eggs are resistant to UV and chemical disinfection, as they are encapsulated by a tough, protective cell wall. In addition, some bacteria are able to regenerate after as little as 24 hours after exposure to UV radiation. These bacteria contain enzymes that repair their DNA after UV damage in a process known as photoreactivation (in the presence of light) or without light (Ellis, 1991; Wegelin et al., 1994). For this reason Gadgil and Shown (1997) suggested using the water within a reasonable time period after disinfection with UV.

Generally there are four classes of disinfection methods utilised for rainwater harvesting, which include chlorination, filtration, UV, and pasteurisation. This review will focus on appropriate treatment systems for smaller quantities of water.

1.7.1 Chlorination

Chlorination is the most common form of disinfection. The addition of chloride to water is practised in numerous countries internationally as it is considered relatively cost-effective (Water for People, 1997). In addition, one of the major advantages of using chlorine, is that the residual chlorine is able to disinfect re-contaminated waters. The amount of residual chlorine available is however dependent on the, chlorine demand, Biological Oxygen Demand (BOD) and the pH of the water. Conversely a major disadvantage of using chlorine is that it needs to be continuously added to the water in order to have an effect. The concentration of chlorine that needs to be added can vary, and it is rather difficult to determine, as the dose depends on several factors. For example, higher doses are required to rid the water of cysts and eggs (approximately 2 – 100 mg/min/L), whereas a dosage of approximately 0.04 – 3 mg/min/L reduces viruses and bacteria (Feachem et al., 1983; WHO, 1996). This value is calculated in parts per million (ppm) and is achieved by multiplying the residual (mg/L) by the exposure minutes (mg/min/L). The dose of chlorine increases when the waters' turbidity and pH increases, but the dose of chlorine decreases roughly ten-fold when the waters' temperature increases by 20°C. The disadvantages of using chlorine becomes apparent when there is a high concentration of organic-matter as this may result in a high chlorine demand, moreover, chlorine is known to react with organic matter which leads to the formation of unwanted organochlorine compounds (Servais et al., 1994).

1.7.2 Filtration

In designing a filtration system or filter membrane it is appropriate to know the size of the contaminants such as bacteria (0.5 – 2 µm), viruses (20 – 80 nm), protozoan cysts (4 – 20 µm) and helminths (0.03 – 2 mm), as the size ranges and diseases associated with water borne pathogens commonly found in water could differ (Burch et al., 1998).

1.7.2.1 Sand filtration

Filters are divided into two types, those requiring pressure and those that rely on gravity. Pressure filters generally consist of a closed steel vessel that contains layers of sand or other granular material through which water is forced under pressure. These filters are generally used in industrial situations for example, public water supplies. Gravity filters generally consist of an open or closed box (usually made of concrete) that is filled with a filter medium such as sand and contains a drain at the bottom. The practice of slow sand filtration is popular amongst non-governmental organisations and is widely applied in rural areas in the developing world and small towns in the developed world (Rooklidge et al. 2005). One benefit of slow sand filters is that they can be designed relatively inexpensively and do not require expensive equipment such as pumps since water filtration relies on gravity. Schulz and Okun (1984) explained that the water runs through about 100 cm of sand, slow enough to allow the growth of a biological film (the schmutzdecke). The schmutzdecke or biofilm layer consists of numerous organisms such as threadlike algae, plankton, diatoms, protozoa, rotifers and bacteria. This film serves as a biological filter and water flows through the sand and biofilm at a relatively slow flow rate in order to allow the biological film to effectively remove microbial contaminants. The sand-bed then allows for absorption, due to electrical forces, chemical bonding and mass attraction that, to date, are not fully understood. In addition, the increased surface area of the sand particles aid in the absorption process, as in one cubic meter of filter sand there will be an average of 15 000 m^2 of surface area (WHO, 1974).

Mwabi et al. (2011) surveyed four household treatment systems namely, the bio-sand filter (BSF), bucket filter (BF), ceramic candle filter (CCF) and the silver-impregnated porous pot filter (SIPP) for water treatment. The four systems were assessed based on the flow rate and the effective removal of physicochemical contaminants such as turbidity, fluorides, phosphates, chlorophyll a, magnesium, calcium, and nitrates. Microbial analysis included the monitoring and removal of *E. coli*, *Vibrio cholerae*, *Salmonella typhimurium* and *Shigella dysenteriae.* For the analysis, water samples were spiked with each contaminant and filtered through each system. The flow rates were measured at 171 l/h, 167 l/h, 6.4 l/h and 3.5 l/h for the BSF, BF, CCF, and SIPP, respectively. The authors found that 99.9% of the fluoride was removed but only 26 – 56% of the magnesium was

removed and overall the bucket filter was the most effective in removing chemical contaminants. The mean percentages of microbial pollutants recorded also ranged between 97% and 100%. The SIPP filter was also the most effective in removing microbial contaminants (99 - 100%), whereas the BF and the BSF only removed 20 – 40% and 20 – 60% of the microbial contaminants, respectively. The authors noted that extensive studies will need to be completed before either of the systems can be proposed to communities for household treatment of drinking water. It was however highlighted that one of the disadvantages of relying on slow sand filters is that every time the biofilm is removed, it requires a period of several days to "ripen" and during this period the filter does not effectively remove bacteria. To counteract this disadvantage, it is recommended that multiple filters be used in parallel.

1.7.2.2 Nanofiltration, nanofibres and applications

The membrane filtration process has been developed to separate a wide selection of particles based on size and molecular weights. According to Baker et al. (2004) a membrane is a discrete, thin interface that moderates the permeation of species that come into contact with the surface. Four membrane filtration techniques exist for the treatment of water. These include microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF) and Reverse Osmosis (RO). Nanofiltration is a passive process operating at pressures as low as 350 – 1000 kPa (Kilduff et al., 2004). This is a process that combines Donnan exclusion and sieving separation, resulting in a molecular weight cut-off of between 300 and 100 Da (Eriksson, 1988). Nanofiltration has been shown to reject monovalent ions by 70% and lowers colour and organic carbon by 90 – 95%. However, one of the major obstacles in applying NF in the treatment of drinking water is fouling, as this lowers the performance of the membranes (Kaiya et al., 1996). Membrane fouling is caused by the build-up of inorganic and organic species and biological activity on the surface of the membrane. This in turn, leads to a decrease in the performance and flux of the membrane, and increases operating costs and shortens the durability of the membrane (AWWA, 1992).

1.7.2.2.1 Electrospinning

The scientific, technological and industrial interest in 1-dementional (1D) nanostructures has increased in the past few years, as they have been shown to have many applications, including air and water filtration (Gibson et al., 2001; Gopal et al., 2006), drug delivery (Hong et al., 2010; Okuda et al., 2010), tissue engineering and in the fields of regenerative medicine (Grafahrend et al., 2001; Koh et al., 2008). Numerous fabrication and synthesis methods exist, including polymerisation against porous templates, self-assembly, and melt-blowing. Recent advances have also allowed for the fabrication of nanofibres through electrostatic spinning or electrospinning, and

this technology not only allows for the up-scaling of production but it is classified as a relatively simple technique (Persano et al., 2013). The benefits of being able to up-scale the electrospinning process includes the ability to produce nanofibres of extreme length (up to km) (Frenot and Chronakis, 2009), the increased surface-area and tunable porosity (Bognitzki et al., 2001; Luu et al., 2003; Zhang et al., 2005; Welle et al., 2007), the intrinsic three dimensional (3D) structure (Pagliara et al., 2009; Sambaer et al., 2010; 2011; Dhandayuthapani et al., 2012) and the incorporation of functional properties (Yang et al., 2012). The eletrospinning process occurs in the presence of an intense electric field, typically ranging from 10^5 to 10^6 $V.m^{-1}$, with this electric field formed between the spinneret and a conductive collector. A uni-axial elongated jet, which is ejected from the surface of a charged polymer solution, having enough molecular entanglements, is then captured on a rolling target electrode. In tailoring the specific properties and functionalities of electrospun products many materials and solvents may be combined. Electrospinning apparatus range from laboratory scale setups, that produce single polymer jets, to industrial scale machines, that produce numerous polymer jets simultaneously. At a laboratory scale many companies offer different spinning and collecting electrode devices and accessories (Persano et al., 2013).

1.7.2.3 Biological Granular Activated Carbon filters

Waters contaminated with phenols, volatile acids, aromatic and aliphatic organics as well as waters requiring BOD and COD removal are generally treated using powdered activated carbon (PAC) (Aktaş and Çeçen, 2007). Granular Activated Carbon (GAC) filters have primarily been used to remove dissolved organic matter by absorption and have proved to be efficient in the treatment of wastewater (Servais et al., 1994; Abu-Salah et al., 1996). Powdered activated carbon and GAC can then be distinguished from each other based on the size of the particles, for example, the average PAC particle sizes range from 15 to 25 µm and GACs are formed from crushed coal or shell granules, or appear in the form of pellets that have been made from powders and binders such as coal tar pitch. Many studies have also shown that bacteria are able to colonise GAC and this has many applications in water treatment processes as the colonised bacteria help significantly in the removal of organic matter (Rittmann and Snoeyink, 1984; McFeters et al., 1987). As the bacteria are able to colonise the external surface and the macropores of the GAC particles it effectively results in biological activated carbon (BAC) that is biofilm-covered GAC. Powered activated carbon is used in powdered activated carbon treatment (PACT) and is a process whereby PAC is added to activated sludge. Both PAC and GAC have to be regenerated, as over time, the particles lose absorption capacity. However, PACs are usually discarded rather than regenerated as regenerating PACs involve the separation of activated carbon particles from colloidal particles. Granular activated carbon, however, may undergo processes such as thermal and hot air oxidation

or bioregeneration (renewing of activated carbon by microbial activities) processes, as generally GAC does not require regular expensive regeneration (Aktaş and Çeçen, 2007).

Projects involving the treatment of rainwater have mainly focused on using GAC filters in combination with another treatment process. For example, in a pilot scale study performed by Kus et al. (2013) over a period of 120 days, a GAC filter followed membrane filtration. The GAC filter was utilised to lower turbidity, dissolved organic carbon (DOC) as well as the heavy metal lead, all of which were noted to not to be within the 2004 Australian Drinking Water Guidelines (NHMRC, 2004) in the harvested rainwater. The treatment system then lowered all the above mentioned parameters to below the ADWG limits namely, the turbidity levels (0.3 – 0.4 NTU), the concentration of DOC (< 0.2 mg/L) and lead (< 0.005 mg/L). Moreover, Naddeo et al. (2013) designed a process of filtration adsorption disinfection (FAD), which included GAC for absorption and UV for disinfection of harvested rainwater. The removal of turbidity, chemical oxygen demand (COD), biological oxygen demand (BOD), DOC, UV_{254}, *E. coli* and total coliforms were monitored during the study. Results indicated that the parameters were reduced significantly using the FAD process (99.9% *E. coli* removal) compared to the GAC process (59.8% *E. coli* removal) when tested separately.

1.7.3 Solar disinfection (direct UV contact)

Solar disinfection (SODIS) proposes numerous advantages. The system offers a point of use treatment, where potable water sources are not present and water needs to be stored. Solar disinfection makes use of, for example, polyethylene terephthalate (PET) bottles that are placed in the sun for a certain number of hours, with these bottles surrounded by different backing surfaces that enable the water to heat up. There has been no indication that photoproducts or additives from the PET bottles contaminate the water, while the bottles are exposed to the sun and which reduces the health risk (Wegelin et al., 2001; Amin and Han, 2009). To enhance the efficiency of SODIS, different backing surfaces that vary in shape and size have been used. Two examples of SODIS treatment systems are represented in Figure 1.1. These devices were introduced in Kenya and Ethiopia for cooking. In summary the foil reflectors direct sunlight onto a dark pot which is enclosed in a clear polypropylene bag. The dark pot essentially converts the sunshine into heat which is then trapped in the bag and one is able to cook food. It was discovered that on a hot sunny day, the plastic bag was not needed and the black pot was able to heat water to pasteurisation temperatures (Safapour and Metcalf, 1999).

The inactivation of bacteria recorded with a SODIS system can vary from no inactivation to a 3-log decrease in 1.5 hours (Wegelin et al., 1994; Reed, 1997; Sommer et al., 1997). The variability in the

results can be as a result of, for example, the transparency of the container, water turbidity, aerobic or anaerobic conditions and the amount of solar radiation received (Odeyemi et al., 1988; Mackenzie et al., 1992; Reed, 1997). Some authors have made use of additional catalysts to enhance the efficacy of SODIS, for example titanium dioxide disinfection for faecal coliforms removal (Watts et al., 1995) and *E. coli* (Zhang et al., 1994) and lemon and vinegar disinfection for the reduction and removal of total coliforms and *E. coli* (Amin and Han, 2011).

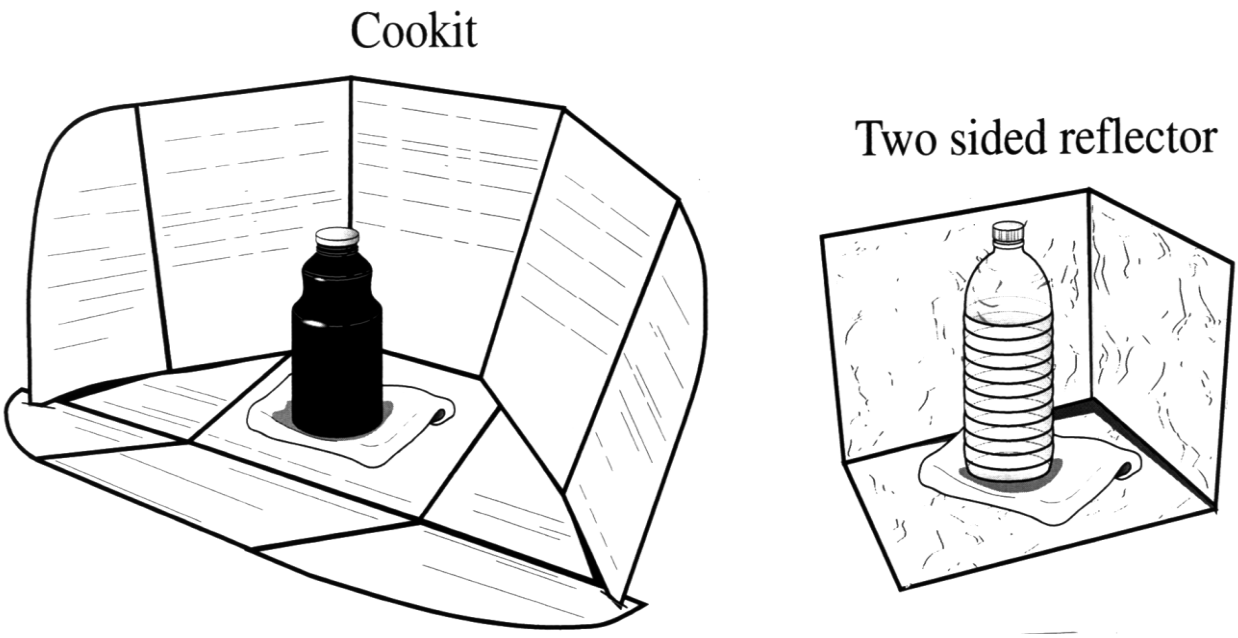

Figure 1.1. Example of two solar water treatment systems that rely on direct exposure of water to sunlight with the use of reflectors to enhance the absorption of sunlight (Safapour and Metcalf, 1999).

Amin and Han (2009) made use of a SODIS system to disinfect rainwater. Rainwater samples were stored in PET bottles that were placed in a solar collector disinfection (SOCO-DIS) system and by determining total and faecal coliforms along with *E. coli* and heterotrophic plate counts, even under moderate weather conditions, it was noted that the rainwater was fully disinfected.

Recently, Ahmed et al. (2013) monitored the effects of ambient meteorological conditions on the inactivation of faecal indicator bacteria (FIB). The reduction of FIBs namely *E. coli* and *Enterococcus* spp. inoculated into slurries of possum faeces and placed on catchment areas in the shade and in the sun, were monitored using culture based methods in a rainwater harvesting system. It was noted that both FIB numbers significantly decreased under sunlight conditions (2 hours) compared to shade conditions (9 – 53 hours). The climatic conditions differed for the two catchment areas, for example solar exposure was higher (16.9 MJ/m^2) during the sunlight roof experiment compared to the shade roof experiment (12.6 MJ/m^2), which contributed to the inactivation discrepancies of the two FIBs placed in the two catchment areas.

1.7.4 Thermal disinfection: Solar Pasteurisation and boiling (indirect sunlight contact)

To date, the US Environmental Protection Agency (USEPA, 2006) and the World Health Organisation (WHO, 1993) have stated that to kill disease- causing microorganisms present in water, the water must be vigorously boiled for 1 min. However, most of the populations that are unable to acquire safe water are the economically impoverished, and boiling the water using electricity, can be costly for most households (Reiff et al., 1996; Metcalf, 1999). Even though Jones and Thompson (1996) state that paraffin is the fuel of choice, traditionally, households use firewood for heating water, but this has resulted in environmental degradation, especially in heavily populated areas, due to the energy source being exploited. Solar radiation offers an abundance of renewable energy, and is readily available in South Africa. The distribution of solar radiation intensity on a global scale is indicated by Acra et al. (1984) where the authors show four belts around the earth. The most favourable belts lie between the 15˚ and 35˚ latitudes. Fortunately, in the southern latitudes it covers the whole of Southern Africa. In the South African White Paper on the Promotion of Renewable Energy, the Council for Science and Industrial Research (CSIR) and Eskom reported that based on available total solar radiation, South Africa yields a yearly average of insulation of between 6000 and 9500 MJ/m^2 (Figure 1.2) (DME, 2002). Page-Shipp (1980) showed that the rest of Southern Africa averaged a yearly insulation of between 7000 and 9500 MJ/m^2.

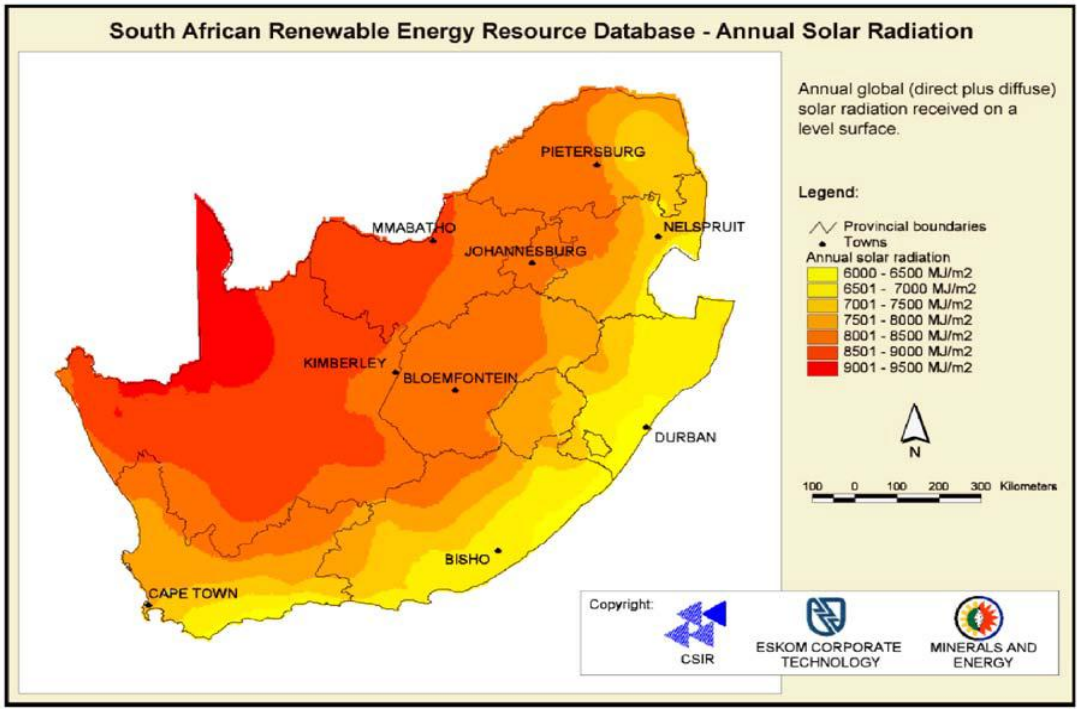

Figure 1.2. Annual solar radiation indicated for South Africa (DME, 2002)

Thermal disinfection of water simply involves heating it to a specific temperature for a specific amount of time. Higher temperatures are effective for shorter time periods. Optimally at 100˚C,

most enteric viruses, bacteria and parasites are inactivated (Ciochetti and Metcalf, 1984). Safapour and Metcalf (1999) have shown how at temperatures of 65˚C, nearly all enteric pathogens were inactivated within minutes to hours. Table 1.5 summarises the volume of water that is produced at various temperatures during one hour. Ghetany and Dayem (2010) observed that at 60˚C, 171 L of clean water was produced per m^2 of solar collector, and these values were reduced to 39 L/m^2 when the water reached 90˚C. These values correspond to 81.5 and 1.1 L/m^2 per kWh of incident solar radiation, respectively.

Pasteurisation has been extensively used in the milk industry, where the liquid is heated to 62.8˚C for 30 min (Metcalf, 1999). The microbial community in water versus milk is different but the majority of the microbial populations are also not heat resistant. In addition, Burch and Thomas (1998) showed how at temperatures as low as 50˚C with sufficient exposure time, bacterial numbers will decrease. The authors also showed that at higher pasteurisation temperatures the exposure time decreases by a factor 10 for every 10˚C increase in temperature.

Table 1.5 The amount of water previously recorded for an hour at various temperatures

Temperature (˚C)	Duration	L/m^2 for each kWh of incident solar energy	Reference
80	hourly	3.61	Bansal et al. (1988)
70	hourly	2.851	El-Ghetany and El-Seesy (2005)
70	hourly	3.181	El-Seesy (2007)
60	hourly	81.5	El-Ghetany and Dayem (2010)
90	hourly	1.1	El-Ghetany and Dayem (2010)

1.7.4.1 Water heating systems

According to Nieuwoudt and Mathews (2005) the technology of heating water to below boiling temperature has gained much interest and for this reason the design and implementation of the heat based disinfection systems is fairly advanced. Currently, there are three types of water heating systems that are manufactured predominantly for domestic use (Solar Energy Equipment, 2000; SANS 1307, 2003). The first and most expensive system is a split system of two components, a collector and a storage tank where water is heated directly or indirectly. The collector is usually installed on the north-facing area of the roof, with the storage tank inside the roof. This allows for a thermo-siphon effect whereby water is able to circulate through the collector due to the ranges of temperatures. This is a passive system and for this reason an electric pump for circulating water is not required. In the second closed coupled system, a flat plate collector that can heat water directly or indirectly and a separate elevated storage tank is attached to the end of the collector. It has been noted that these systems are less expensive and installation is easier than the split systems. Lastly, a less efficient closed system, is the integrated collector storage, or the integral collector (ICS)

systems comprising of a collector that is used to heat and store the water. These systems are the most cost effective. Close-coupled systems are also usually placed on the north-facing section of pitched roofs. For this, both the flat plate collector and the storage tanks are exposed. Being a passive system, again the water moves via the thermo-siphon effect (Nieuwoudt and Mathews, 2005).

1.7.4.2 Factors that influence the performance of Solar Water Heating systems

Factors that influence the performance of ICS and Solar Water Heating (SWH) can be divided into five major categories, and are examined here according to Nieuwoudt and Mathews (2005).

One of the major factors influencing the efficiency of the solar water heater is the inclination and the orientation of the system. The angle from the horizontal position of the solar water heater is defined as the inclination. The horizontal postion refers to the collector facing vertically upwards. The direction that the inclined collector faces is known as the orientation. Solar heating flat plate collectors are generally contrived from high conductivity materials such as copper, aluminium, steel and combinations thereof (Solar Energy Equipment, 2000). Polymer materials have also been used, particularly in ICS solar heaters (Mathews and Rossouw, 1997; Faiman et al., 2001; Cristofari et al., 2002). The advantages of using polymer materials include factors such as, reduced cost and scaling, and these polymers have been shown to have increased freezing and corrosion resistance. However, disadvantages compared to the metals are the lower temperature limitations, lower thermal conductivity and the polymers tend to deteriorate over extended periods of time due to UV exposure, even with UV protection filters. Once the water has been heated, it is perhaps useful to maintain the temperature increasing the retention time of treatment (in the case of pasteurisation) and if the water is to be used for other domestic uses such as bathing it will again be useful to maintain the desired temperature. For two component and closed-coupled solar water heaters, insulted cylindrical tanks are frequently used. If the system does not make use of thermo-siphoning to treat the water, the drop in temperature during the night is usually negligible. The effects of inadequate insulation of the storage tank are quite dramatic and have a major effect on the whole systems performance (Mathews and Rossouw, 1997). It is important to use a glaze on solar water heater collectors as many studies have shown that it limits the percentage of heat lost when the wind blows. The effect of wind on heat loss is the same if the wind is blowing at only 2.3 m/s or less than 10 km/h on non-insulated heat collectors (Mathews and Rossouw, 1997; Nabilek et al., 1999). To date the most cost-effective glazing for solar water heater collectors is glass, which possesses a transmittance of up to 90% (Solar Energy Equipment, 2000). Another good material for collector glazing is polycarbonate as it also exhibits a 90% transmittance with the added advantage that it is

resistant to scratching and, in contrast to glass, can withstand impact (Solar Energy Equipment, 2000; Chaurasia and Twidell, 2001; Fasulo et al., 2001).

1.8 Conclusions

Inadequate water supplies and poor sanitation services impact the socio–economic status of individuals and communities (Mara, 2003; Moore et al., 2003; Montgomery and Elimelech, 2007; Johnson et al., 2008). Rainwater harvesting has been shown to provide a cost effective manner in which water for potable and domestic purposes can be provided. In addition, the rainwater may be used for other activities such as irrigation, car washing and toilet flushing. Besides using harvested rainwater as an alternative water source during water scarcity, other benefits of using rainwater harvesting include the ability to decentralise water collection systems. In doing so, the burden of diseases associated with drinking contaminated water sources is eliminated. Moreover, by harvesting large quantities of rainwater, the demand on municipal water supplies will be lowered and will allow for a reduction in storm water run-off. Most importantly, rainwater harvesting has the potential to increase the sanitation levels and thus allow for improved public health in rural areas (Mwenge Kahinda et al., 2007; Ahmed et al., 2011a).

Water quality is thus of the essence in determining whether the water source is fit for the intended purpose. The assessment of water is defined as "the determination of the sources, dependability, extent, and quality characteristics of water resources, on which is based an evaluation of the possibilities for their utilisation and control," (UNESCO/WMO, 1988). Depending on the atmospheric pollution, the harvesting method and the storage of rainwater, the quality of harvested rainwater may fluctuate and be compromised by chemical and microbiological contamination as a result of, for example, roof runoff that was in contact with bird or animal droppings entering the tank (Fewtrell and Kay, 2007). It is therefore not surprising that studies have indicated that the quality of rainwater is often not suitable for drinking purposes (Yaziz et al., 1989; Zhu et al., 2004; Sazakli et al., 2007).

The treatment and safe storage of rainwater utilised potable and domestic purposes have gained much attention in recent years. Many studies have shown that relatively inexpensive treatment systems can improve the microbial quality of rainwater and in turn lower the risks of illness and death even in situations where poor sanitation is evident (Sobsey, 2002; Murcott, 2006; van Halem et al., 2009). Various treatment methods are utilised for the removal or reduction of chemical and microbial contaminants in rainwater thus exist, and include chlorination, filtration, UV, and pasteurisation. From a social point of view, many authors have noted that in order for treatment systems to work and continue working effectively, community involvement and the development of

infrastructure is imperative (World Bank, 1993). In addition, when deciding on the appropriate treatment system, from a technical point of view, access to supplies, spare parts and training will strongly influence the choice of treatment.

1.9 Aims of study

The aims of this study included the monitoring of the chemical and microbial quality of rainwater from domestic rainwater harvesting tanks (DRWH) in a sustainable housing development in Kleinmond, South Africa. The chemical and microbial parameters were compared to drinking water standards stipulated by the various national and international guidelines and based on these results the second aim of the study was to use chemical and microbial parameters to evaluate the efficiency of four household point-of-use treatment systems, namely, activated carbon, slow sand filtration, PVA nanofibre column and a activated carbon/PVA nanofibre column, for the treatment of harvested rainwater. The last aim was to determine the efficiency of a solar pasteurisation system in heat treating rainwater at different temperature ranges. For this chemical and microbial paramenters were monitored at the various temperature ranges.

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Chemical and Microbial Quality of Domestically Harvested Rainwater in Kleinmond, South Africa

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Abstract

Rainwater harvesting has gained popularity worldwide as it provides an accessible and free water supply directly to the consumer. As many advantages have been associated with the use of domestically harvested rainwater, the aim of this study was to ascertain the primary chemical and microbiological quality of harvested rainwater in a peri-urban region of South Africa during the low and high rainfall periods. Rainwater samples were collected from domestic rainwater harvesting tanks (DRWH) in a sustainable housing development in Kleinmond, South Africa, on eight occasions from 29 tanks during the period of March to August 2012. The chemical and microbial parameters were compared to drinking water standards including the South African and Australian Quality Guidelines. The rainwater quality was within all the chemical standards (cations, anions, metal ions, pH and temperature) analysed for potable water, with the concentration of organic matter (COD) ranging between 4 mg L^{-1} to 9.5 mg L^{-1}. However, the total coliform (90%), *Escherichia coli* (60%), enterococci (7.9%), faecal coliform counts (37.9%) and heterotrophic bacteria (96%) counts obtained significantly exceeded ($p < 0.05$) the stipulated guidelines. The microbial analysis results thus concurred with international studies that have indicated that the harvested rainwater was not fit for potable use, with limited application for domestic purposes, without treatment.

Key Words: Rainwater harvesting; chemical quality; indicator organisms

2.1 Introduction

Rainwater harvesting is practised worldwide and refers to the collection and storage of rainwater runoff for domestic and agricultural use (Gould, 1999; Mwenge Kahinda et al., 2008; Helmreich and Horn, 2009). Domestic rainwater harvesting (DRWH) can also potentially serve as a safe and inexpensive water supply for households. In the United Kingdom approximately 400 rainwater harvesting systems are implemented annually and the government has also encouraged the implementation of these systems through its Code for Sustainable Homes (Rainwater Harvesting, 2008; Environment Agency, 2010). During 2006 the Australian government initiated a subsidy scheme for residents in the Queensland State that utilised harvested rainwater for non-potable domestic uses (Ahmed et al., 2011a). Domestic rainwater harvesting has thus been recognised as an alternative water resource by a number of governmental organisations (Lee et al., 2010; Australian Government, 2011; Rowe, 2011) and favourable government policies are directly related to the increase in harvested rainwater usage. However, currently only 0.4% of the households in South Africa utilise rainwater harvesting tanks (Statistics South Africa, 2010). Domestic rainwater harvesting has thus been earmarked by the Department of Water Affairs as a strategy to improve water availability amongst especially the rural communities lacking sufficient water supply infrastructure. Consequently the South African government, through the Department of Water and Affairs and Forestry (DWAF) and the Department of Science and Technology (DST) has made the distribution of tanks possible throughout five provinces i.e.; Eastern Cape, Limpopo, KwaZulu-Natal, the Free State and the Western Cape (De Lange, 2006).

Depending on the atmospheric pollution, the harvesting method and the storage of rainwater, the quality of harvested rainwater may fluctuate and be compromised by chemical and microbiological contamination as a result of, for example, roof runoff that was in contact with bird or animal droppings entering the tank (Pathak and Heijnen, 2000). Numerous studies have thus indicated that the quality of rainwater is often not suitable for drinking purposes (Yaziz et al., 1989; Zhu et al., 2004; Sazakli et al., 2007). Inadequate water supplies and poor sanitation services impact on the socio–economic status of individuals and communities (Mara, 2003; Moore et al., 2003; Montgomery and Elimelech, 2007; Johnson et al., 2008). Water quality is thus of the essence in determining whether the water source is fit for the intended purpose and the potential risks and hazards that are associated with rainwater harvesting thus need to be understood by likely users.

There are however, no international or national guidelines for routine rainwater analysis and monitoring. In certain instances local authorities have developed suitable manuals and guidelines for harvested rainwater use. For instance in Austin, Texas, the Texas Water Development Board in

cooperation with other parties, published the third edition of The Texas Manual on Rainwater Harvesting in 2005. The purpose of the guide is to educate the public on rainwater harvesting and its applications. The authors describe the quality and treatment of harvested rainwater and the cost estimations and financial incentives involved in the implementation of DRWH systems (Krishna et al., 2005). In addition, there is no legislation in South Africa regarding the use of rainwater, as the potential public health risks associated with untreated rainwater have not been identified (Mwenge Kahinda et al., 2007).

It would, however, be impractical to assess rainwater sources for all possible pathogen contaminants and for this reason many studies have investigated for the presence of indicator organisms (De Kwaadsteniet et al., 2013). To allow for a reliable indication of the potential risks of infection, a combination of indicators, such as total coliforms (TC), faecal coliform (FC), *Escherichia coli* (*E. coli*) and enterococci (ENT), are routinely analysed. Indicator organisms are used as an index of the potential presence of entero-organisms as they are present in high numbers in the intestinal flora of humans and warm-blooded animals and are found in faecal matter. The presence of the indicator group of organisms in water also indicates that the water has been faecally polluted and is not safe for domestic use prior to disinfection due to the possible presence of pathogens (Rompré et al., 2002).

The primary microbial and chemical contaminants present in DRWH tanks during the low and high rainfall periods was investigated in a sustainable housing scheme established in Kleinmond, a coastal town in South Africa. The results were compared to the South African Water Quality Guidelines (DWAF, 1996), the South African National Standards for Drinking Water (SANS, 2005), the World Health Organisation (WHO, 2011) and the Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011) to assess the quality of harvested rainwater.

2.2 Materials and Methods

2.2.1 Sample Site and Collection

The Kleinmond Housing Scheme (Western Cape, South Africa), initiated in 2010 by the Council for Scientific and Industrial Research (CSIR) and the Department of Science and Technology, was used as the sampling site. The 40 m^2 houses, situated in an urban coastal area, are part of the Government's initiative to provide low cost sustainable development houses in South Africa and are fitted with alternative technologies such as solar panels and rainwater harvesting tanks (De Villiers, 2011). From a cluster of 411 houses, 29 houses were selected for sampling rainwater during the study period (March to August 2012). The selection process was conducted by consulting with an official from the Kleinmond Municipality.

A map of the cluster of houses used in this study is depicted in Figure 2.1, with the houses sampled throughout the study indicated by black circles. Green circles indicate the houses that were replaced with alternative, new houses (red circles) that were sampled from the third and sixth sampling sessions (Table 2.1). The DRWH tanks connected to these houses became unavailable for sampling during the study period due to unforeseen circumstances. As can be seen from the map, there are no obstacles obstructing the roofs, i.e. trees or electrical power lines. While the risk of contamination may still occur, the lack of apparent areas (no trees) for birds and other animals to nest in, may be an added advantage in lowering the risk for the contamination of the rainwater (Ahmed et al., 2011b; 2012a, b).

The vertical, polyethylene rainwater tanks, have a capacity of 2000 litres and were installed at the housing sites at the end of 2011 (Figure 2.1) and the tanks were therefore less than a year old at the time of sampling. The catchment area consisted of concrete roof tiles, namely double roman standard plus. No first flush diverters were installed to eliminate the first flush of debris from the roof surface into the tanks.

Figure 2.1. A map of the Kleinmond Housing Scheme (Western Cape, South Africa). GPS coordinates: 34˚20’11.81” S 19˚00’59.74” E. The houses selected for sampling throughout the study are indicated by black circles. Green circles indicate the houses that were replaced with alternative, new houses (red circles) that were sampled from the third and sixth sampling sessions.

During the low rainfall period sampling was conducted every three weeks (March to May 2012) and thereafter one to four days after a rain event [high rainfall period (June to September 2012)]. Table 2.1 indicates the dates samples were collected from the rainwater tanks at the Kleinmond Housing Scheme.

Table 2.1. Sampling dates for the study period March to August 2012

Sampling Session	Date
1	5 March 2012
2	28 March 2012
3	19 April 2012
4	22 May 2012
5	5 June 2012
6	19 June 2012
7	7 August 2012
8	21 August 2012

The house and sampling numbers were recorded as indicated in Table 2.2. It should be noted that, as indicated in Table 2.2, for sample numbers 8 and 28, the sampling of the rainwater tank at house 8390 was replaced with house 8395 (19 April – third sampling session) and house 8352 was replaced with house 8351 (19 June – sixth sampling session) as indicated by the red circles in Figure 2.1, respectively, due to unforeseen circumstances.

Table 2.2. Sample numbers correlating to the house numbers at the Kleinmond pilot plant used in this study

Sample Number	House number	Sample Number	House number
1	8220	16	8399
2	8217	17	8402
3	8216	18	8473
4	8212	19	8404
5	8208	20	8466
6	8506	21	8408
7	8387	22	8344
8	8390 replaced with 8395	23	8339
9	8392	24	8345
10	8498	25	8337
11	8497	26	8347
12	8394	27	8335
13	8494	28	8352 replaced with 8351
14	8477	29	8332
15	8401	30	Control

For the microbial and chemical analysis, water samples were collected in 2 L sterile polypropylene bottles that had been sterilised with 70% ethanol, rinsed with tap water and stored on ice to maintain a low temperature. The temperature and pH of the rainwater at the sampling locations were measured using a hand-held mercury thermometer and colour-fixed indicator sticks with a pH range

of 0 - 14 (ALBET®, Barcelona, Spain). Rainfall patterns were obtained from the South African Weather Services (SAWS, 2012). In total eight sampling sessions were conducted for the duration of the study with a total rainfall recorded for each month. Control water samples (i.e. treated municipal water) were collected from the Kleinmond Water Treatment Plant for the first, seventh and eighth sampling sessions.

2.2.2 Chemical Analysis

Metal and anion concentrations were determined for the first sampling session. For the determination of the metal concentrations, Falcon™ 50 mL high-clarity polypropylene tubes containing polyethylene caps, were pre-treated with 1% nitric acid before sampling. The concentrations of metals such as aluminium (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn), amongst others, were determined. Metal concentrations were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) according to Saleh et al. (2000) and nitric acid digestion. All chemical analyses were performed at the Central Analytical Facility (CAF), Stellenbosch University.

Anions detected included chloride (Cl), nitrate (NO_3) and sulphate (SO_4). High-Performance Ion Chromatography (HPIC) was used to determine the concentration of anions. The concentration of organic compounds in the water samples was also determined for the last sampling session. Water samples were sent to the CAF, in order for the Chemical Oxygen Demand (COD) to be determined for each rainwater sample.

2.2.3 Enumeration of Total Heterotrophic Bacteria and Faecal Indicators

Various conditions and media were used to enumerate *Escherichia coli*, total coliforms, faecal coliforms, enterococci and total heterotrophic bacteria (Table 2.3). Each medium was prepared according to the manufacturer's instructions. Samples were processed within 4 hours of sampling in order to obtain accurate total coliform and *E. coli* counts.

For each of the 29 tanks and control samples, an undiluted and diluted (10^{-1}) rainwater sample was spread plated onto various media as indicated in Table 2.3. Nine millilitres of 0.9% NaCl was used for the serial dilution with an inoculum of 1 mL. Subsequently 100 µL of the dilution series samples were spread plated onto ChromoCult® Coliform Agar (CCA) (Merck, Biolab, Wadeville, Gauteng) to obtain total coliform and *E. coli* numbers after the plates were incubated at 35 ± 2°C for 18 - 24 hours. For the enumeration of faecal coliforms and enterococci the samples were plated onto the various media within 36 hours of sampling. For faecal coliforms a serial dilution was prepared as

mentioned above with 100 µL aliquots spread plated onto m-FC Agar (Merck) whereafter the plates were incubated at 44.5 ± 0.5°C for 22–24 hours. From the second sampling session onwards enterococci were enumerated in the same manner as the faecal coliforms, however 100 µL of each dilution of each sample was additionally spread plated onto Slanetz and Bartley Agar (Oxoid, Hampshire, England) and incubated at 36 ± 22°C for 44-48 hours.

Table 2.3. Media and conditions of incubation for the identification of indicator organisms

Organism/s	Medium	Temperature	Duration of Cultivation (hours)
Heterotrophic Plate Count	Nutrient Agar (Merck)	35 ± 2°C	18 – 24
Total Coliforms	m-Endo Agar (Merck) ChromoCult® Coliform Agar (CCA) (Merck)	35 ± 2°C	18 - 24
Faecal Coliforms	m- FC Agar (Merck)	44.5 ± 0.5°C	22–24
Enterococci	Slanetz and Bartley Agar (Oxoid, Hampshire, England)	36 ± 22°C	44-48

In order to analyse the general microbial quality of the rainwater, total heterotrophic bacteria were enumerated through the pour plate method. A serial dilution of each sample was made (as indicated previously) and 1 mL of each dilution, for each sample, was added to Nutrient Agar (NA) (Merck) plates which were then incubated at 37°C for 18 - 24 hours.

2.2.4 Membrane Filtration

Membrane filtration was used to obtain total coliform counts and the procedure was performed in duplicate within 4 hours of sampling. For sampling sessions one and two, undiluted samples were filtered, but no single colonies were visible as the sample plates were overgrown with bacteria [a Too Numerous to Count (TNTC) value was obtained]. From sampling session three a 1:4 dilution was made of each sample in duplicate. The method consisted of filtering 100 mL (25 mL rainwater sample plus 75 mL sterile distilled water) of each sample through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (10 kPa, 10 psi). The filters were then incubated on m-Endo Agar (Merck) at 35 ± 2°C for 18 - 24 hours (Table 2.3) (U.S. Environmental Protection Agency, 2009).

2.2.5 Statistical Analysis

The data obtained from the microbial and chemical analysis of the collected rainwater samples was assessed using the statistical software package Statistica™ Ver. 11.0 (Stat Soft Inc, Tulsa, USA). In each data set, analysis of the residuals revealed that the data was not normally distributed, which

pointed to the requirement for the Spearman Rank Order Correlation as non-parametric correlation technique to test the significance of the data set. In this test, a Restricted Maximum Likelihood solution (REML) with a type III decomposition was performed on all data recorded to establish whether or not there was variation between sampling sessions. Once it was established that variation was indeed present, Variance Estimation, Precision and Comparison (VEPACK) analysis was performed, however the data for pH, temperature and average rainfall were set as fixed variables and time and sample were set as grouping variables. Data pairs that showed significant differences were subsequently further analysed using the Least Squares Difference (LSD) test and probabilities for Post-hoc pair-wise comparisons. Data that did not present variation was not analysed using this method. For example, data obtained for faecal coliforms was analysed by applying the Repeated measures ANOVA, using Holm's Sequential Bonferroni method. In all hypothesis tests, a significant level of 5% was used as standards (Dunn and Clark, 1974). In all tests a P-value smaller than 0.05 was considered as statistically significant.

2.3 Results and Discussion

2.3.1 Rainfall, Temperature and pH

The overall rainfall patterns recorded for Kleinmond during the sampling period were obtained from the South African Weather Services (2012). Initially sampling sessions were conducted every three weeks, and once the rainfall events had started to increase (during the high rainfall season), sampling sessions were performed three to four days after a rain event. Table 2.4 indicates the total rainfall for each month (March to August) during the sampling period.

Table 2.4. The total rainfall for each month (March to August) during the sampling period.

Month	Total rainfall (mm) per month during the Sampling Period
March 2012	16.8
April 2012	56.5
May 2012	30.6
June 2012	74.7
July 2012	90.7
August 2012	198.1

As indicated in Table 2.4 the total monthly rainfall (mm) pattern observed for sampling periods 1 to 4 (ranging from 16.8 mm in March to 30.6 mm in May) was lower than for sampling periods 5 to 8 (ranging from 74.7 mm in June to 198.1 mm in August). An increase of water and debris flowing into the tanks during the fifth to eight sampling sessions would thus have been expected. It was also expected that during this time period, the contaminants, such as debris, bird droppings etc., that would have accumulated over the summer and autumn months would be washed into the tanks

during the rainy season (no first flush diverters were installed on the tanks). As the rain continued to fall during the rainy season, the bacterial numbers could have either decreased due to the inflow of rain diluting the water in the tanks and so diluting the amount of bacteria, or increased due to added debris, collected during the winter months, being flushed into the tanks.

The temperature (Table 2.5) and pH levels (Table 2.6) of the rainwater samples decreased slightly with each sampling, when compared to the previous sampling's data, although this difference was not significant ($p > 0.05$). There was also no significant difference ($p > 0.05$) observed between the temperature and pH levels of the rainwater collected from the 29 tanks during sampling two to eight. The pH ($R = -0.655$) and temperature ($R = -0.705$) of the rainwater samples also showed a significant negative correlation to the total rainfall data obtained. This implies that as the rainfall increased, the pH and temperature decreased accordingly.

Table 2.5. Mean temperature results of rainwater samples collected from the Kleinmond housing scheme

Sampling	Mean	Range
2	23.97±0.74	22 – 25
3	19.66±0.94	18 – 22
4	14.79±1.52	13 – 18
5	14.86±1.48	13 – 18
6	14.28±0.84	13 – 16
7	12.59±0.68	11 – 14
8	13.59±1.09	11 – 15

The SANS 241 (2005), DWAF, (1996) and ADWG (NHMRC and NRMMC, 2011) guidelines stipulate that drinking water samples should have a pH between 5 – 9.5, 6 – 9 and 6.5 – 8.5, respectively. However, as indicated in Table 2.6 the pH levels recorded from sampling two to eight did not adhere to the recommended guidelines stipulated by DWAF and ADWG, however, the samples were within the SANS 241 (2005) guidelines throughout the investigation.

Table 2.6. Mean pH results of rainwater samples collected from the Kleinmond housing scheme

Sampling	Mean	Range
2	6.02±0.09	6 – 6.5
3	5.49±0.05	5.25 – 5.5
4	5.39±0.32	5 – 6
5	5.39±0.32	5 – 6
6	5.16±0.42	4.5 – 6
7	5.00±0.00	5 – 6
8	5.14±0.30	5 – 6

There is insufficient data to stipulate a health guideline for pH levels, although drinking water with a pH less than 4 and higher than 11 may be detrimental to human health. Water with pH levels lower than 6.5, as in the case of the rainwater samples, can also be corrosive (NHMRC and

NRMMC, 2011). Various studies, locally and internationally have recorded average pH values of between 6 – 8.5 (Nevondo and Cloete, 1999; Zhu et al., 2004; Handia et al., 2003; Sazakli et al., 2007; Al-Salaymeh et al., 2011).

2.3.2 Chemical analysis results

Various anions and metal cations were analysed during the first sampling session. Lithium, Beryllium, Tin and Titanium were not detected in the 29 rainwater samples. However, lithium (7.31 µg/L) was detected in the control sample. All other anions and metal cations analysed were within the DWAF (1996), SANS 214 (2005), WHO (2011) and ADWG (NHMRC and NRMMC, 2011) drinking water guidelines and were present in trace amounts (Table 2.7).

Although no chemicals, that were analysed during this study, were above the recommended guidelines according to SANS, DWAF and the ADWG, elevated values of aluminium (Al) (1220.26 µg/L) and iron (Fe) (232.22 µg/L), were observed for the control sample, collected from the Kleinmond Municipality Water Treatment Plant. The Kleinmond Water Treatment Plant doses the water with an alum compound to act as a flocculant during the water treatment process, which could explain the increased concentration of aluminium observed (Overstrand Municipality, 2011).

The chemical oxygen demand (COD) of the harvested rainwater samples collected during sampling 8 was also determined. The kit used to determine the COD had a detection range of 4 to 40 mg/L. The COD concentration of certain rainwater samples collected from the DRWH tanks in Kleinmond, were however, lower than 4 mg/L and therefore the range of COD values described were between 4 to 9.5 mg/L. No significant difference between the COD of the rainwater samples ($p > 0.05$) was also recorded. A low COD implies that the concentration of organic matter in the harvested rainwater samples is correspondingly low as COD refers to the concentration of oxygen required to oxidise all the organic matter present (SANS, 2005). However, no recommended value for COD is stipulated in the SANS 241 (2005), ADWG (NHMRC and NRMMC, 2011), WHO (2005) and DWAF (1996) guidelines.

Table 2.7. Cation concentrations (mean) obtained for the rainwater samples compared to recommended concentrations as stipulated by the respective drinking water guidelines (n=29).

Cation	Sample Mean	Sample Range	Control	SANS 241	DWAF	ADWG	WHO
Aluminium as Al (µg/L)	78.27±34.07	37.87 – 180.20	1220.26	300	150	100	-
Iron as Fe (µg/L)	19.29±11.31	7.36 – 61.34	232.22	200	100	300	-
Sodium as Na (mg/L)	15.37±2.76	11.81 – 22.27	22.01	200	100	180	-
Calcium as Ca (mg/L)	11.58±0.83	10.52 – 14.19	12.14	150	200	200	-
Zinc as Zn (µg/L)	3.86±3.41	0.57 – 15.77	1.20	5000	3000	3000	-
Barium as Ba (µg/L)	3.67±1.43	1.95 – 7.35	3.71	-	-	2000	700
Silicon as Si (mg/L)	3.42±0.50	2.79 – 4.98	1.52	-	-	-	-
Potassium as K (mg/L)	2.68±0.91	1.75 – 6.31	0.69	50	50		-
Copper as Cu (µg/L)	1.90±0.83	0.75 – 3.73	0.82	1000	1000	2000	2000
Magnesium as Mg (mg/L)	1.60±0.24	1.30 – 2.15	2.27	70	30	200	-
Vanadium as V (µg/L)	0.91±0.19	0.58 – 1.54	0.59	200	1000	-	-
Chromium as Cr (µg/L)	0.80±0.24	0.47 – 1.70	0.38	100	50	50	50
Arsenic as As (µg/L)	0.48±0.11	0.32 – 0.80	0.12	10	10	10	10
Selenium as Se (µg/L)	0.41±0.16	0.11 – 0.79	0.22	20	20	10	40
Manganese as Mn (µg/L)	0.38±0.40	0.11 – 1.82	13.61	100	50	500	-
Lead as Pb (µg/L)	0.18±0.08	0.08 – 0.39	0.05	20	10	10	10
Cadmium as Cd (µg/L)	0.15±0.11	0.06 – 0.58	0.01	5	5	2	3
Nickel as Ni (µg/L)	0.14±0.06	0.07 – 0.32	1.96	150	-	20	70
Strontium as Sr (mg/L)	0.14±0.02	0.10 – 0.18	0.05	-	-	-	-
Mercury as Hg (µg/L)	0.08±0.05	0.03 - 0.19	0.07	1	1	1	6
Molybdenum as Mo (µg/L)	0.08±0.02	0.06 – 0.14	0.02	-	-	50	-
Boron as B (mg/L)	0.04±0.03	0.02 – 0.18	0.01	-	-	4	2.4
Cobalt as Co (µg/L)	0.02±0.01	0.01 – 0.06	0.28	500	-	-	-
Phosphorus as P (mg/L)	0.02±0.01	0.01 – 0.03	0.01	-	-	-	-
Lithium as Li (µg/L)	nd	-	7.31	-	-	-	-
Beryllium as Be (µg/L)	nd	-	nd	-	-	60	-
Tin as Sn (µg/L)	nd	-	nd	-	-	-	-
Titanium as Ti (mg/L)	nd						-

nd – not detected

Nitrite (NO_2), phosphate (PO_4) and fluoride (F) were not detected in any of the rainwater samples (Table 2.8). The concentrations of chloride (Cl^-) ranged from 16.70 mg/L (tank 7) to 29.90 mg/L (tank 14), nitrate concentrations ranged from 1 mg/L (tank 5) to 2.30 mg/L (tank 24) while, sulphate (SO_4) ranged from 3.70 (tank 7) to 19.50 mg/L (tank 14). These obtained values were well below the respective drinking water guidelines. The only anions detected in the control sample were SO_4

(31.4 mg/L) and Cl^- (24.7 mg/L). None of the chemicals analysed in the rainwater samples during this study were above the recommended guidelines according to SANS (2005), DWAF (1996), WHO (2011) and the ADWG (NHMRC and NRMMC, 2011). No fluoride was also detected during this study and fluoride is an important element in drinking water since it reduces dental decay (Satur et al., 2010). However, a study in India showed fluoride concentrations that exceeded the World Health Organisation guidelines resulted in dental and skeletal fluorosis in patients (Hussain et al., 2012). Sazakli et al. (2007) also noted that consumers should be made aware of the low fluoride concentrations in rainwater and should therefore be advised to take a fluoride supplement to prevent dental decay if rainwater serves as the primary potable water source.

Table 2.8. Anions concentrations (mean) obtained from the rainwater samples compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 29).

Anions (mg/L)	Mean	Range	Control	SANS 241	DWAF	ADWG	WHO
Chloride as Cl^-	21.2 ± 3.5	16.7 – 29.9	24.7	200	100	250	-
Sulphate as SO_4	7.95 ± 4.36	3.7 – 19.5	31.4	400	200	250	-
Nitrate as NO_3	1.52 ± 0.31	1 – 2.30	nd	10	6	50	50
Nitrite as NO_2	nd	-	nd	10	6	3	3
Phosphate as PO_4	nd	-	nd	-	-	-	-
Fluoride as F	nd	-	nd	1	1	1.5	1.5

nd – not detected

2.3.3 Microbiological quality of harvested rainwater

Where applicable, the counts obtained in this study for each indicator group were compared to the drinking water standards stipulated by the South African National Standards (SANS, 2005), the World Health Organisation (WHO, 2011), the South African Water Quality Guidelines for Domestic Water Use of the Department of Water Affairs and Forestry (DWAF, 1996) and the Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011).

2.3.3.1 Total Coliforms

Total coliform counts, utilising the spread plate technique, for the first to fourth sampling period (low average rainfall recorded) are represented in Figure 2.2. On average the total coliform counts recorded during this period ranged from 5.96×10^4 CFU/100 mL (sampling one) to 1.03×10^5 CFU/100 mL (sampling four). However, where the rainfall events started to increase, on average the spread plate counts ranged from 1.75×10^4 CFU/100 mL in the fifth sampling period to 5.56×10^4 CFU/100 mL in the eighth sampling period.

Overall the total coliform results obtained by spread plating onto ChromoCult® Coliform Agar for sampling periods one to four were higher than the average total coliform counts obtained in sampling periods five to eight, with no total coliform counts (0 CFU/mL) also recorded sporadically throughout the last four sampling sessions for numerous tanks. For all the rainwater samples collected from the DRWH tanks (1 to 29) for sampling one to eight, 90% of the total coliform counts then exceeded the recommended values as stipulated by the respective drinking water guidelines (DWAF, 1996).

Total coliform counts were also enumerated utilising m-Endo Agar (Merck, Biolab Diagnostics) and membrane filtration (Table 2.9). For the first two sampling sessions, undiluted samples of 100 mL were filtered. The number of total coliforms could, however, not be distinguished as the filters were over grown with bacteria and the values were recorded as > 250 CFU/100 mL (results not presented). From the third to the eighth sampling period a 1:4 dilution was performed and coliform numbers were recorded for most samples by membrane filtration, with the exception of a few rainwater tank samples, where the filters were consistently overgrown with bacteria and results were again recorded as > 250 CFU/100 mL.

For samplings one, two, five, six and seven, all the rainwater samples collected from tanks 1 to 29 yielded total coliform numbers above the standards recommended by DWAF (1996). During sampling three and eight, total coliforms present in 97% of the rainwater samples were above the guidelines, while 79% of the counts were higher than the guidelines during sampling four.

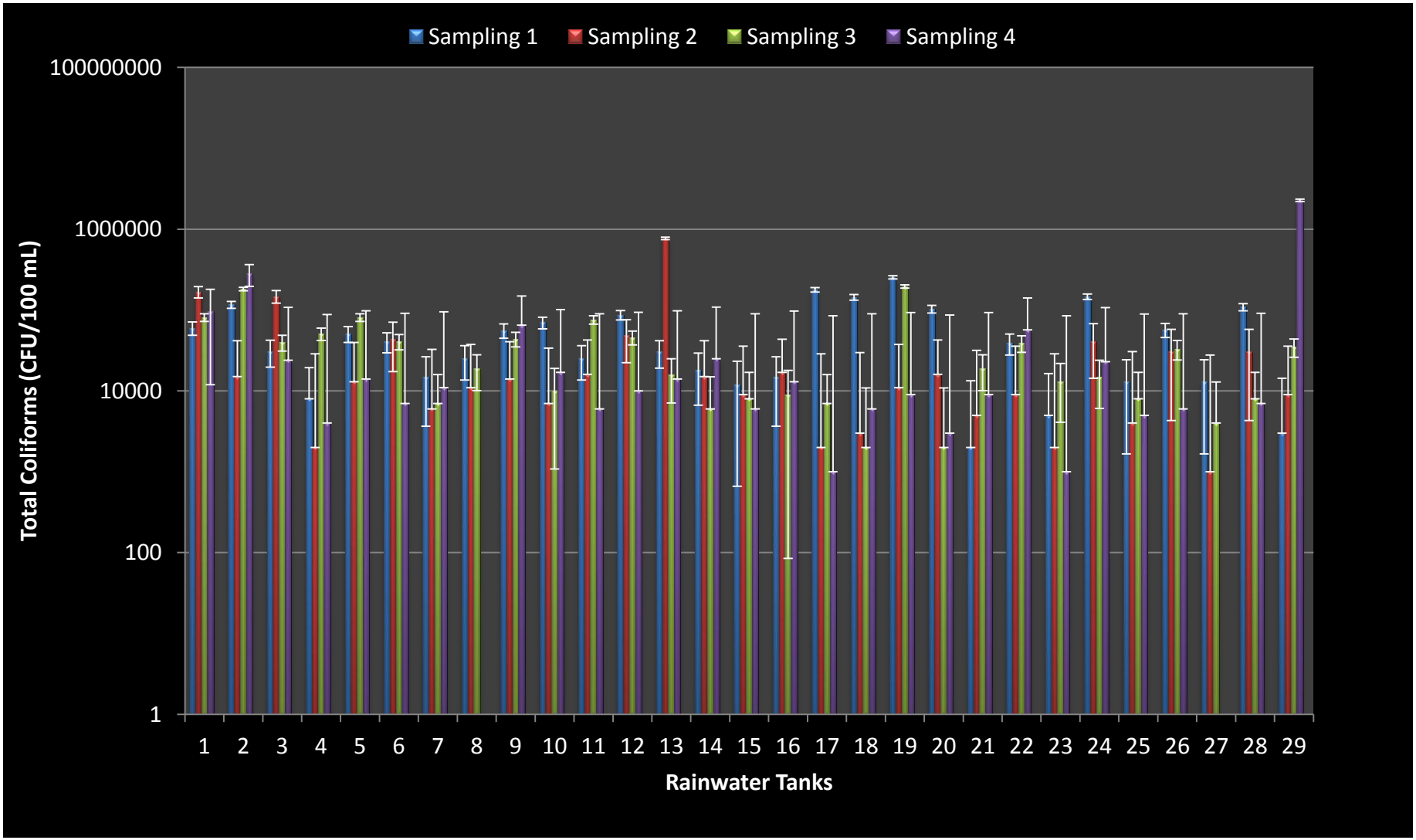

Figure 2.2. Enumeration of total coliform counts (CFU/mL) in the rainwater samples (Kleinmond) obtained for sampling one to four (March to May 2012) utilising the spread plating technique (ChromoCult® Coliform Agar).

Table 2.9. Total coliform counts obtained through membrane filtration for the DRWH tanks sampled in the Kleinmond Housing Scheme

Total Coliforms (CFU/100 mL)						
Sample	**3rd Session**	**4th Session**	**5th Session**	**6th Session**	**7th Session**	**8th Session**
1	368	8	1000	1152	1000	560
2	1000	148	2304	1000	1344	0
3	1080	0	1888	624	960	288
4	416	24	216	1000	1280	124
5	288	0	2176	704	1120	656
6	300	576	2624	1000	964	3456
7	176	20	1024	1056	512	392
8	0	8	1408	672	1000	1024
9	1000	12	1216	800	176	1024
10	1000	768	1504	768	192	24
11	1000	124	56	960	120	20
12	536	408	1280	1216	928	192
13	1000	0	1984	1152	1000	240
14	1000	1056	2944	352	928	416
15	1000	0	208	832	1184	2656
16	1000	112	1760	1024	1024	48
17	24	52	2112	1280	768	1984
18	76	0	2048	1600	240	976
19	656	0	1000	1000	432	624
20	928	724	3264	1056	1440	808
21	976	624	1952	1312	800	144
22	1000	1000	1000	1248	1000	992
23	268	160	1312	480	800	432
24	120	496	496	1024	1000	2304
25	1000	52	52	960	1152	1184
26	1000	1000	1000	960	672	32
27	160	16	16	320	848	432
28	268	24	24	608	120	2080
29	176	1000	1000	1120	576	24

With the exception of a few samples, overall the results recorded for total coliforms utilising the spread plate technique (ChromoCult® Coliform Agar) and membrane filtration (m-Endo Agar) significantly exceeded ($p < 0.05$) the stipulated guidelines (DWAF, 1996). High total coliforms counts indicate that the general sanitary quality of the water is compromised and should not be used for potable purposes (DWAF, 1996). These results correlate to previous studies, conducted on the quality of rainwater, where high total coliform counts were recorded in harvested rainwater samples (Spinks et al., 2006; Lévesque et al., 2008; Al-Salaymeh et al., 2011). Sazakli et al. (2007), found that coliforms were present in 80.3% of all of their rainwater samples analysed, with the authors indicating that microbial and chemical parameters exhibited seasonal fluctuations. From a pilot study, Spinks et al. (2006) found that 90% of their 49 samples analysed were contaminated with total coliforms. They

also hypothesised that there was no significant relationship between the levels of microbial indicator organisms and the use of first flush diverters, cleaning the gutters or cleaning the holding tank.

2.3.3.2 Escherichia coli

The total *E. coli* counts obtained in CFU per 100 mL for sampling one to four (the graph for samplings five to eight is not presented) utilising the spread plate technique with ChromoCult® Coliform Agar are presented in Figure 2.3.

For the first to fourth sampling period, on average the spread plate *E. coli* counts ranged from 2.5×10^3 CFU/100 mL to 2.0×10^3 CFU/100 mL, while during the fifth to eighth sampling periods lower *E. coli* counts were obtained overall with averages ranging from 1.0×10^2 CFU/100 mL (sampling five) to zero *E. coli* detected during sampling eight. Significantly high ($p < 0.05$) *E. coli* counts were also recorded during sampling sessions one and four, with the highest count of 1×10^4 CFU/100 mL recorded for numerous tanks (9, 11, 12 and 29) during sampling four (Figure 2.3). According to the DWAF (1996) ADWG (NHMRC and NRMMC, 2011) and WHO (2011), *E. coli* should not be present in water sources utilised for drinking purposes, however utilising the spread plate technique and Chromocult® Coliform agar, 38% of all the samples exceeded the recommended drinking water guidelines of 0 CFU/100 mL, as stipulated by DWAF (2006), the ADWG (NHMRC and NRMMC, 2011) and the WHO (2011). Results for the DRWH tanks also varied with fluctuating *E. coli* counts obtained during the respective sampling periods and between sampling occasions.

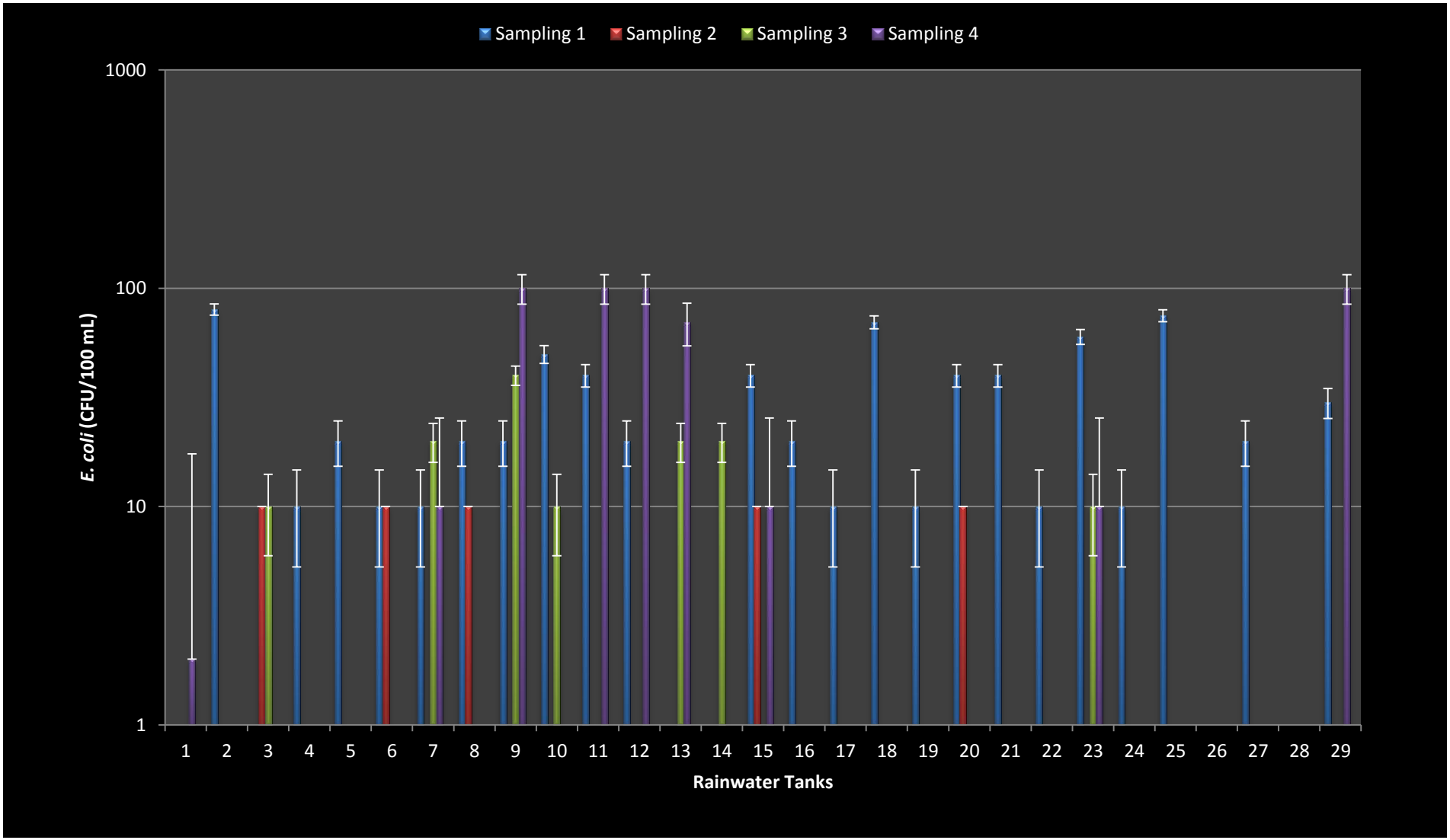

Figure 2.3. Enumeration of *E. coli* numbers (CFU/100 mL) in the rainwater samples (Kleinmond) obtained for sampling one to four utilising the spread plating technique (ChromoCult® Coliform Agar).

The membrane filtration (MF) technique (utilising m-Endo agar) was also utilised to enumerate the *E. coli* counts. The MF technique is a routine monitoring technique applied by local municipalities and water treatment facilities for the monitoring of the microbial quality of drinking water. Compared to the multiple tube fermentation technique, it is more accurate, time- and cost-effective. The inability of the MF to recover coliforms that have been injured or stressed can however be a disadvantage. Exposure to chemical treatment such as chlorine can also cause sub-lethal damage to the cells, preventing the cell forming a colony on the selective media (Rompré et al., 2002).

While low *E. coli* counts were detected in sampling two using ChromoCult® Coliform Agar, no *E. coli* was detected during this sampling period using membrane filtration and m-Endo agar. Throughout sampling one to four numerous tanks also had no *E. coli* present with 23% of the DRWH tanks sampled exceeding the drinking water guidelines. During sampling five to eight, *E. coli* counts ranged from 6.0×10^1 CFU/100 mL to 7.0×10^1 CFU/100 mL, respectively (Figure 2.4). The lowest *E. coli* counts of 0 CFU/100 mL (utilising m-Endo and the membrane filtration technique) were recorded in sampling periods five (tank 7 and 28), six (tank 2) and seven (tank 1 and 8), while the highest count of 2.2×10^2 CFU/100 mL was recorded in sampling five (tank 21). However overall, for sampling five to eight, the highest *E. coli* counts utilising the membrane filtration technique were obtained for sampling eight, while the lowest counts were obtained for sampling seven.

In addition, 96% of the tanks sampled exceeded the recommended *E. coli* count of 0 CFU/mL during this sampling period (five to eight), with 60% of the DRWH tanks sampled overall (sampling one to eight) exceeding the stipulated guideline using the membrane filtration technique.

Generally the *E. coli* results obtained, by spread plating onto ChromoCult® Coliform Agar, for sampling periods one to four, were higher than the average *E. coli* counts obtained during the same sampling period using the membrane filtration technique. The multivariate tests of significance and one way ANOVA revealed significant variation ($p < 0.05$) between *E. coli* numbers utilising the two techniques (membrane filtration and the spread plate technique).

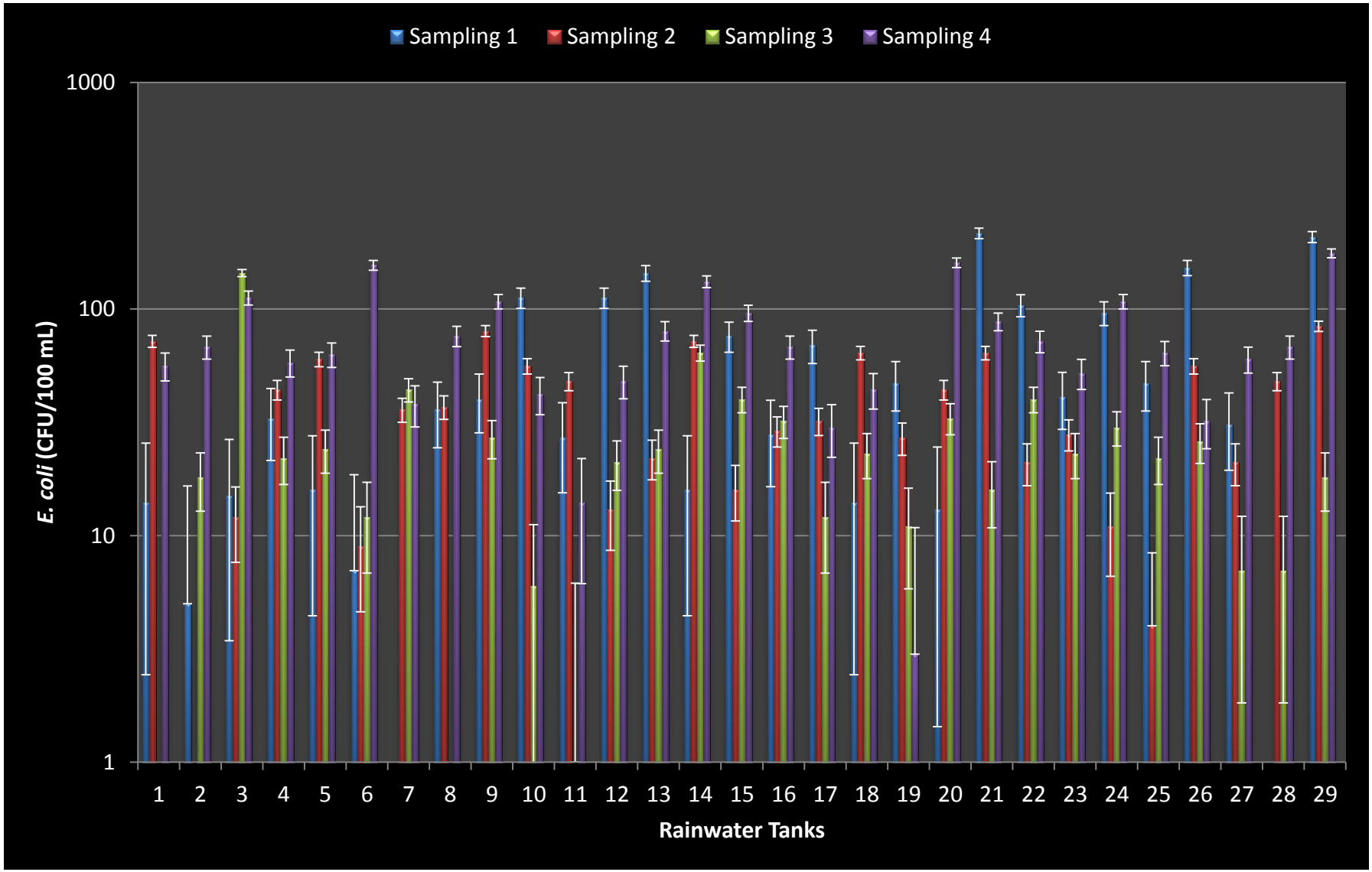

Figure 2.4. Enumeration of *E. coli* numbers (CFU/100 mL) in the rainwater samples (Kleinmond) obtained for sampling five to eight utilising membrane filtration (mEndo agar).

For the entire sampling period (March to August 2012) the lowest average *E. coli* count recorded, using the spread plating technique was obtained in sampling eight (0 CFU/100 mL), while the highest average count was obtained in sampling one (2.5×10^3 CFU/100 mL). While results fluctuated between the sampling occasions, high *E. coli* counts were also recorded for tanks 9 and 29 (sampling one to eight). In contrast, for the membrane filtration technique, the lowest average *E. coli* count was obtained in sampling two (0 CFU/100 mL) with the highest average count obtained in sampling eight (7.0×10^1 CFU/100 mL). While the *E. coli* results also fluctuated between sampling occasions, the highest count was similarly recorded for tank 29 (sampling one to eight).

Escherichia coli is included as a specific indicator organism of faecal pollution from warm-blooded animals (Pinfold et al., 1993; Rompré et al., 2002; Sazakli et al., 2007). This study therefore suggests that the water should not be used for drinking purposes as faecal contamination from warm-blooded animals may be present in the harvested rainwater tanks (DWAF, 1996). The enumeration of *E. coli* in numerous studies also varied, with Spinks et al. (2006) indicating that *E. coli* was present in 33% of the samples analysed, Ahmed et al. (2012a) indicating that *E. coli* was present in 63% of the collected rainwater samples and Albrechtsen (2002) finding *E. coli* in 79% of the samples tested. Ahmed et al. (2012a) also found that wild animals, such as possums and birds could be the main contributors to faecal contamination in DRWH tanks.

2.3.3.3 Faecal Coliforms

As total coliforms do not necessarily represent the contamination of faecal origin, the presence of faecal coliforms (FC) (also referred to as thermotolerant coliforms) was also monitored, with the results obtained for the rainwater samples collected from tanks one to twenty-nine, for sampling two to eight represented in Figure 2.5. For the second to eighth sampling period, on average the spread plate counts for FC ranged from 6.9×10^1 CFU/100 mL to 1.2×10^3 CFU/100 mL, respectively. For numerous tanks throughout the entire sampling period, no faecal coliforms were recorded, while the highest count of 2.8×10^4 CFU/100 mL was recorded in sampling seven (tank 21).

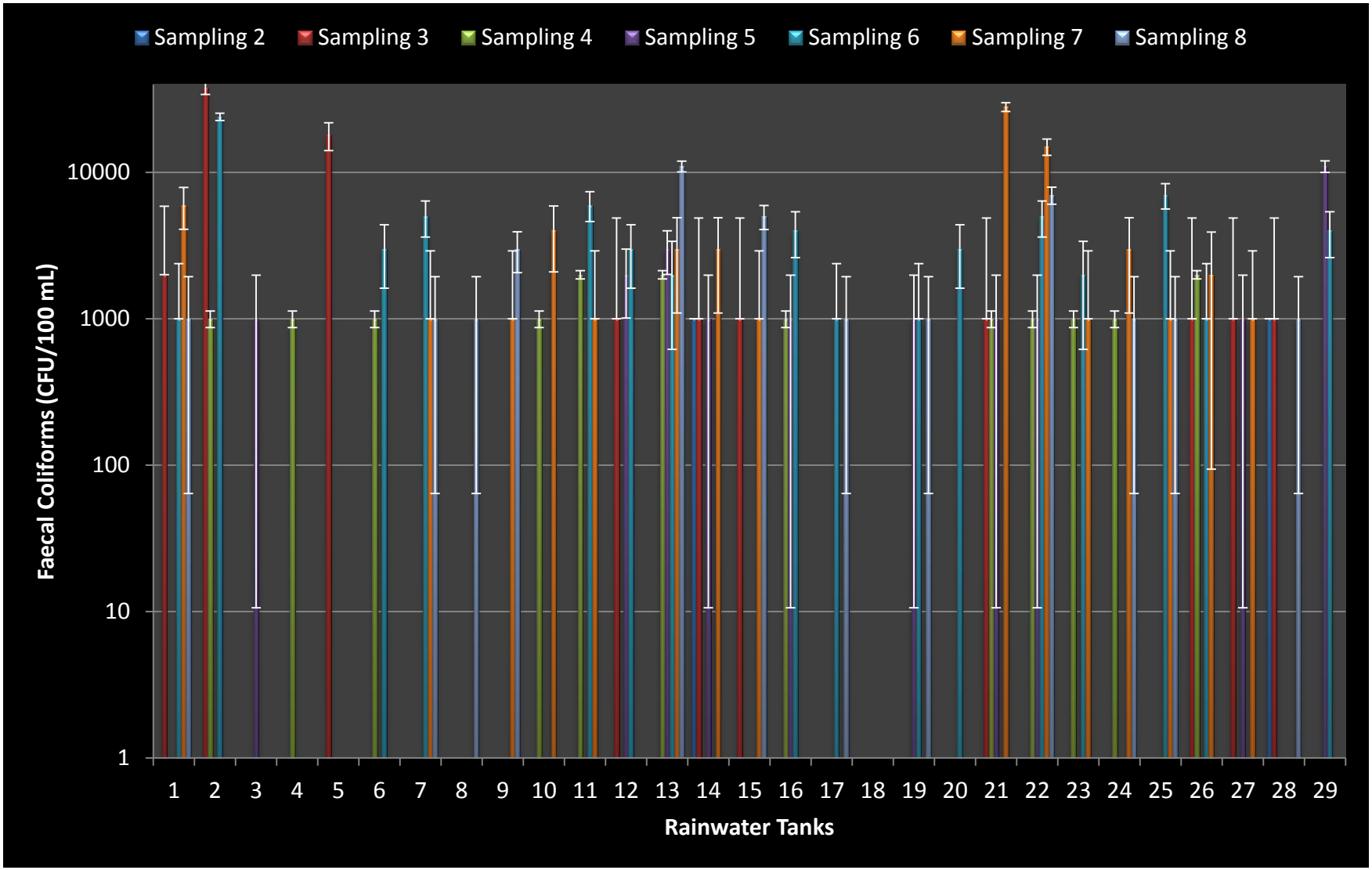

Figure 2.5. Enumeration of faecal coliform numbers (CFU/mL) in the rainwater samples (Kleinmond) obtained for sampling two to eight (March to August 2012) utilising the spread plating technique (m-FC Agar).

The drinking water standards, according to DWAF (1996), the South African National Standards for drinking water (SANS, 2005), WHO (2011) and ADWG (NHMRC and NRMMC, 2011) stipulate that there should be no faecal coliforms present if the water is to be used for potable purposes. While during the second sampling, the majority of the tanks had no faecal coliforms present, 6.9% of the rainwater samples did not conform to the standards stipulated by DWAF (1996), SANS 241 (2005), WHO (2011) and the ADWG (NHMRC and NRMMC, 2011) with FC numbers exceeding acceptable levels. Of the rainwater samples analysed in sampling three and four, 34.5% and 41.4% had FC numbers that did not comply with the respective drinking water guidelines, while during the fifth, sixth, seventh and eighth sampling periods 34.5%, 55.2%, 51.0% and 41.4%, respectively, of the samples collected from the rainwater tanks had faecal contamination above the stipulated standard.

A total of 37.9% of the rainwater tanks thus contained elevated faecal coliform numbers (numbers above the standards as mentioned previously), which implies that these tanks may possibly be contaminated with faecal pollution and are therefore not suitable for potable purposes (DWAF, 1996). A study conducted by Despins et al. (2009) in Canada however, found that only 14% of 360 samples analysed had FC contamination and observed that cold weather significantly improved the microbial quality of harvested rainwater. Similarly, Handia et al. (2003) also found that in their study conducted in Zambia, 14% of their samples showed elevated levels of FC from rainwater stored in ferrocement tanks.

2.3.3.4 Enterococci

Enterococci results were obtained by spread plating and culturing the rainwater samples on Slanetz and Bartley Agar (results not presented). No enteroccoci counts were recorded for samplings two, five and six and very few tanks contained significant enterococci counts in sampling three, seven and eight, with the highest average count of 8.9×10^2 CFU/100 mL recorded in sampling four. Enterococci should not be present in water samples according to guidelines stipulated by DWAF (1996) and the ADWG (NHMRC and NRMMC, 2011). During samplings three and four 3.4% and 34.5% of the rainwater samples exceeded this specified drinking water guideline count, while in sampling seven and eight, 6.9% and 10.3% of the samples exceeded the enterococci standards, respectively.

Enterococci, including predominantly faecal streptococci, originate from human or animal faeces. Therefore enterococci serve as an indicator of faecal pollution but are present in lower

numbers than total and faecal coliforms. Based on the results obtained the faecal streptococci contamination in numerous DRWH tanks sampled in the Kleinmond Housing Scheme was thus below the stipulated guidelines (DWAF, 1996; NHMRC and NRMMC, 2011) as on average only 7.9% of the rainwater tanks sampled during March to August 2012 had elevated numbers of enterococci present. These numbers were however, lower than the enterococci counts recorded in harvested rainwater samples in many other studies (Spinks et al., 2006; Ahmed et al., 2008; Ahmed et al., 2012c). For example Ahmed et al. (2012c), found that 83% of 100 DRWH tanks sampled did not conform to enterococci standards set for drinking water purposes as the rainwater samples were contaminated with various *Enterococcus* species including *E. faecalis, E. mundtii, E. casseliflavus, E. faecium, E. hirae, E. avium,* and *E. durans,* all of which contained virulence genes.

2.3.3.5 Heterotrophic Plate Count

The Heterotrophic Plate Count (HPC) results obtained by pour plating and culturing the rainwater samples on Nutrient Agar (NA) are represented in Figure 2.6 (sampling one to eight). Repeated measures ANOVA was then used to analyse and compare results obtained for HPC.

For the first to fourth sampling period, on average the pour plate counts ranged from 6.8×10^4 CFU/100 mL in the first sampling period to 4.6×10^5 CFU/100 mL in the fourth sampling period. Overall, for sampling one to four, the highest average HPC counts utilising the pour plate technique were obtained for samplings four while the lowest counts were obtained for sampling one. For sampling period five to eight, on average the counts ranged from 6×10^4 CFU/100 mL in the fifth sampling period to 4.3×10^5 CFU/100 mL in the eighth sampling period. Overall, for sampling five to eight, the highest average HPC counts were obtained for sampling eight while the lowest counts were obtained for sampling five.

The DWAF (1996) guidelines stipulate that the heterotrophic bacterial count should not exceed 100 CFU/mL. During the first sampling, 100% of the rainwater samples exceeded the standards stipulated by DWAF (1996). The percentage of rainwater samples where the HPC count exceeded the acceptable levels, as stipulated by the respective guidelines, were 100%, 93.1%, 100%, 79.3%, 100%, 100%, and 96.6% for the second, third, fourth, fifth, sixth, seventh and eight sampling, respectively.

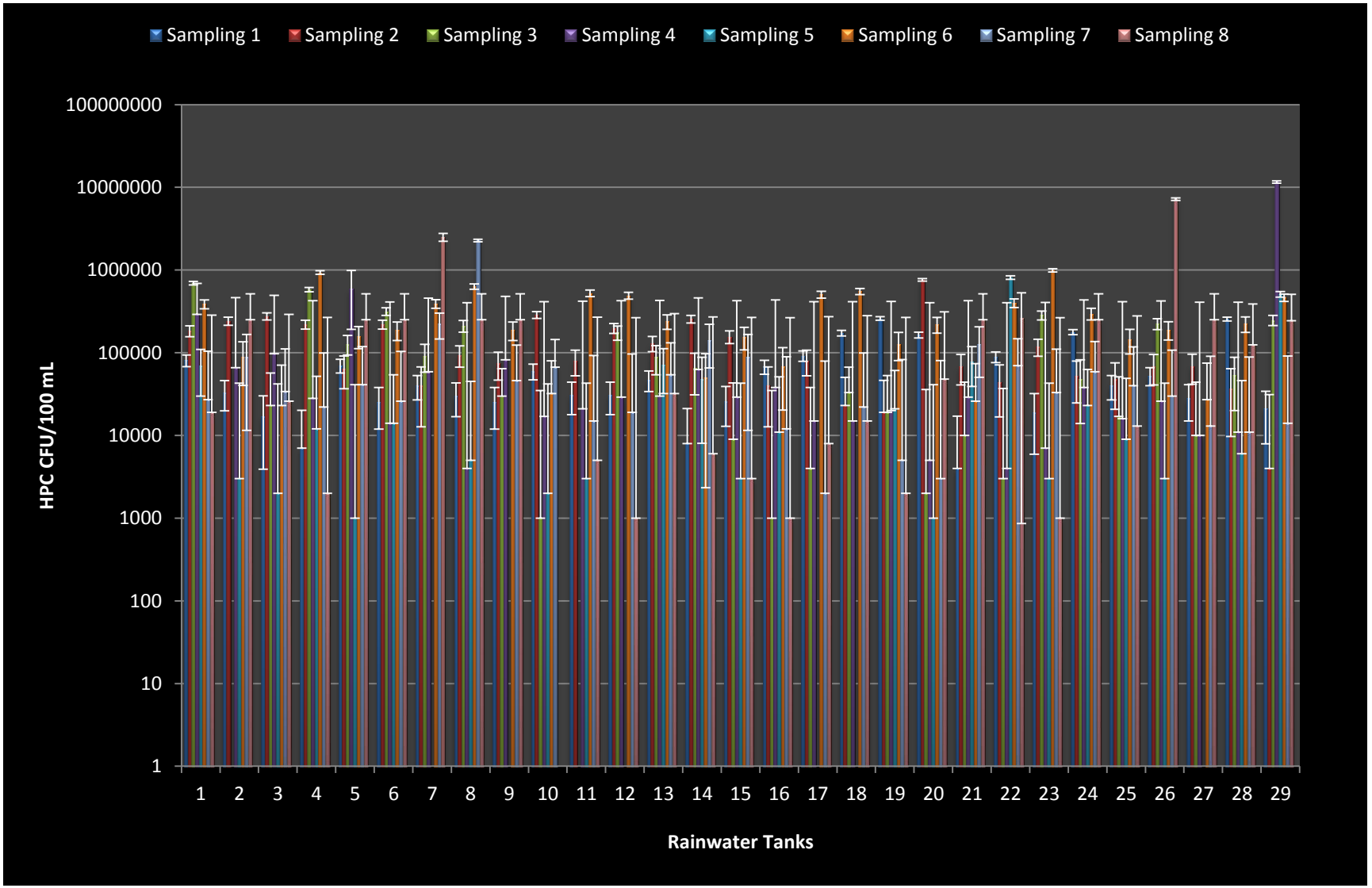

Figure 2.6. Enumeration of heterotrophic bacteria (CFU/100 mL) in the rainwater samples (Kleinmond) obtained for sampling one to eight using the pour plate technique (Nutrient Agar).

During this study 96.1% of the rainwater tanks sampled thus exceeded the drinking water standards due to elevated heterotrophic bacterial numbers recorded. Even though the percentage of HPC numbers that exceeded the guidelines was lower during sampling five in comparison to the other seven sampling sessions, the differences between HPC values recorded for all sampling sessions was not significant ($p = 0.52$). With the exception of sampling five, on average the heterotrophic numbers in the present study were comparable to a number of previous studies conducted. Evans et al. (2006) found that all of the 67 rainwater samples collected had an elevated HPC with the same results observed by Albrechtsen (2002), Uba and Aghogho (2000) and Lye (1987) where again, all of their samples contained elevated numbers of heterotrophic bacteria.

2.3.4 Correlations between indicators and physico-chemical properties of rainwater

A significant correlation ($p = 0.00$) could be established between total coliforms (utilising membrane filtration) and the following parameters: *E. coli* counts (utilising membrane filtration) ($R = 0.30$), *E. coli* counts (utilising the spread plate technique) ($R = - 0.15$), faecal coliforms ($R = 0.29$), temperature ($R = - 0.19$), pH ($R = - 0.40$), and rainfall ($R = - 41$).

After analysing all the data, the REML and the Fixed Effect test yielded significant variations ($p = 0.00$, $F = 16.83$) amongst the eight sampling sessions for total coliforms (utilising membrane filtration). For this reason an LSD test was performed that showed that the same highest mean differences (-1089.28 ± 133.9726) ($p = 0.00$) were observed between sampling sessions one and five, and two and five, while the lowest mean difference with the least amount of variation for total coliforms was recorded between sampling sessions seven and eight (15.31 ± 133.97) ($p = 0.909$) as indicated in Figure 2.7.

Significant correlations ($p < 0.05$) were also noted between *E. coli* counts, utilising the spread plate technique and the following parameters: *E. coli* counts, utilising membrane filtration ($R = -0.21$), enterococci ($R = 0.15$) and rainfall ($R = -0.36$). After analysing all the data the REML and the Fixed Effect test showed significant variation ($p = 0.00$, $F = 8.968$) for *E. coli* counts (utilising the spread plate technique) amongst the eight sampling sessions. The LSD test then showed that the highest mean difference (2465.52 ± 449.86) ($p = 0.00$) was recorded between sampling sessions one and seven, while the lowest mean difference, with the least amount of variation, was recorded between sampling sessions two and six (68.97 ± 449.8644) ($p = 0.878$).

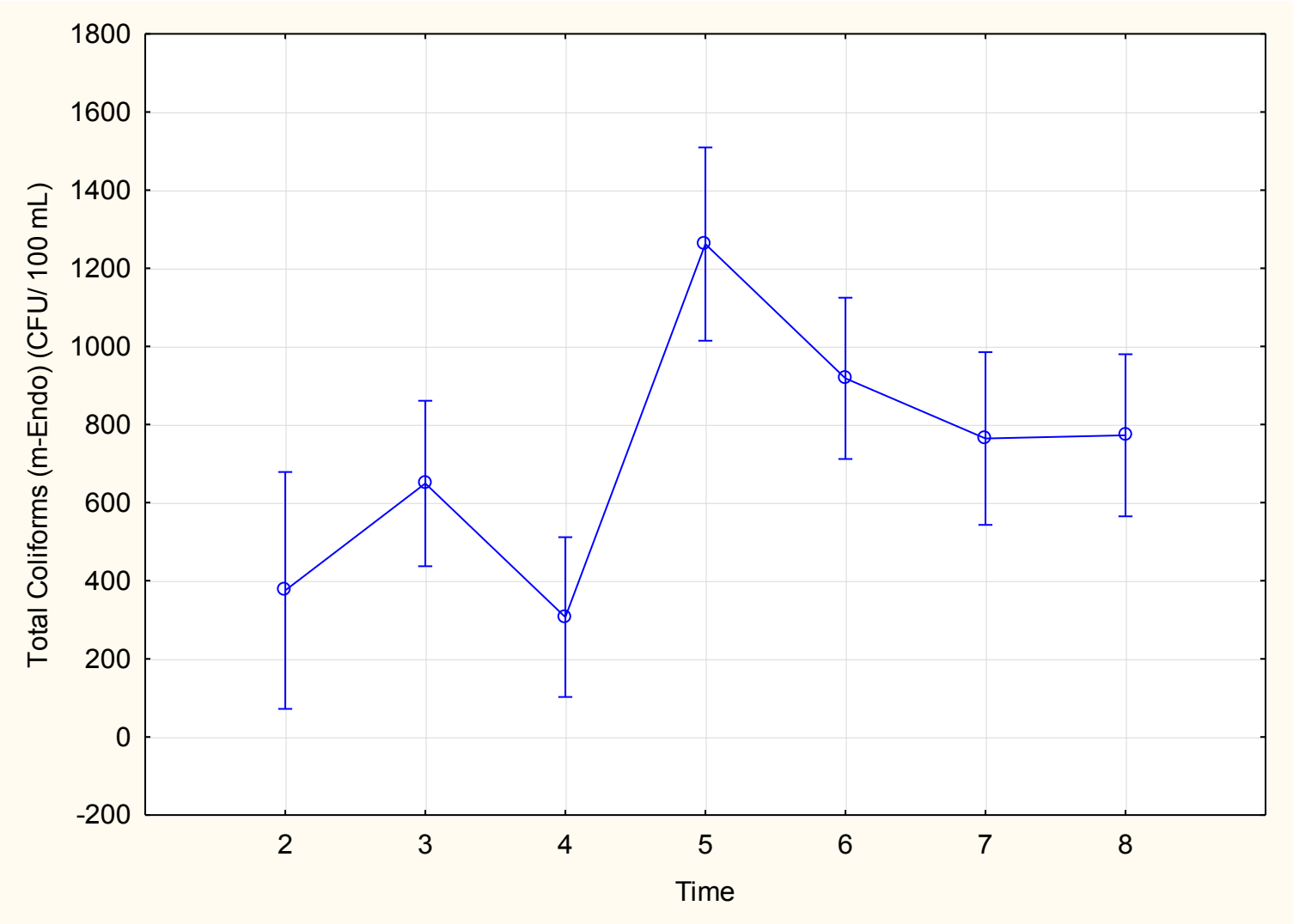

Figure 2.7. The results of an LSD test indicating the significant differences between mean total coliform counts (m-Endo Agar) over a period of eight sampling events.

Enterococci (R = - 0.194), temperature (R = - 0.313, p = 0.00), pH (R = - 0.424, p = 0.00) and rainfall (R = 0.61, p = 0.00) also exhibited a significant correlation to *E. coli* counts (utilising membrane filtration). Over time, using the REML and the Fixed Effect test, the data exhibited significant variation (p = 0.00, F = 17.005) amongst the eight sampling sessions. An LSD test showed that the highest mean difference (-73.103 ±9.40) (p = 0.00) was recorded between sampling sessions three and seven, while the lowest mean difference with the least amount of variation was recorded between sampling sessions one and two (1.86± 9.4)(p = 0.843).

Statistical analysis also revealed significant correlations (p = 0.00) between faecal coliforms and the other parameters such as total coliforms (utilising membrane filtration) (R = 0.29), temperature (R = - 0.25) and pH (R = - 0.236). After analysing all the data the Repeated Measures ANOVA and the Bonferroni test showed no significant variation for faecal coliforms (p = 0.06, F = 2.94) amongst sampling sessions four, seven and eight. The rest of the sampling sessions were not analysed in this manner due to a lack of variation within the data. As mentioned previously, enterococci showed significant correlations to *E. coli* counts utilising membrane filtration and the spread plate technique, pH (R = 0.15, p = 0.04), and rainfall (R = -0.19, p = 0.01). Due to the lack of variation within the data, no further analysis was performed.

As in many groundwater environments, the organic carbon, electron acceptors and many other critical nutrients such a nitrogen and phosphorus may be present in low concentrations in rainwater. As contaminants are continuously washed into the rainwater tanks, carbon may become available to the microbial consortia within the rainwater tanks, which may utilise the available carbon for growth and energy. However, as inorganic nutrients may not be present in excess, organisms capable of extracting them from, for example, soil contaminants may have an added competitive advantage (Rogers et al., 2001).

According to the Spearman Rank Order Correlations, no significant correlation could be determined between COD and the microbiological indices (p > 0.05). The monitoring of treated wastewater and the treatment efficiency is generally defined by measuring global parameters such as Biological Oxygen Demand (BOD), COD, Total Organic Carbon (TOC) and Total Suspended Solids (TSS) (Thomas et al., 1997; Wacheux, 1998). The BOD gives an indication of waste biodegradability in biological treatment processes and is defined as the potential oxygen removal by aerobic heterotrophic bacteria able to utilise organic matter (Brookman, 1997). However, there are many pitfalls associated with the BOD test, for example, as the test is performed over five days, acquiring information is slow. The test is also labour intensive as it requires dilutions and other manipulations which take a long time to complete. However, most importantly it is well documented that even

though the BOD test is still used, it remains insensitive and inaccurate at low concentrations (Khan et al., 1998). For these reasons, it was decided that the in order to determine the organic pollution, the COD would be measured. The sources of pollution for rainwater are not as diverse as for example sewage effluent and for this reason it was not surprising that COD of the rainwater samples was not as high as for example treated sewage final effluent samples where for example in the Eastern Cape, South Africa, Igbinosa and Okoh (2009) reported COD values for wastewater effluent that ranged from 34.82 and 238.00 mg/L. As mentioned previously, the kit used to determine the COD of rainwater in this study ranged from 4 – 40 mg/L and 48.3% of the rainwater tanks had COD values below the detection range of the kit, it is therefore speculated that a significant correlation could not be determined between COD and microbiological indices due to insufficient data.

All the data sets with significant correlation between the microbiological indicators and various metals and anions in the rainwater samples were recorded (Table 2.10). The most significant inverse correlation was established between selenium and *E. coli* counts obtained from membrane filtration ($p = 0.01$), with a negative Spearman's correlation coefficient of -0.437. Selenite, an oxyanion of selenium, can influence *E. coli* counts in the rainwater by inactivating proteins, blocking DNA repair and interfering with cellular respiration (Turner et al., 1998). It has also been shown that selenium can be utilised in many metabolic pathways, for example the synthesis of macromolecules such as tRNA, formate dehydrogenase enzymes and many other proteins (Pinsent, 1954; Böck et al., 1991; Burk, 1991).

Significant negative correlations were also observed between total coliform counts and the presence of the ions, silicon, vanadium, chromium and sulphate in the harvested rainwater samples. These negative correlations could be due to certain bacterial metabolic pathways requiring ions for example, silicon (Si) is the second most abundant element in soil and exists in plants in concentrations comparable to macronutrients such as calcium, magnesium and phosphorus. In grasses, silicon is often present at higher concentrations than other inorganic constituents (Epstein, 1999). Some bacteria are known to accumulate silicon in their membranes (Heinen, 1967). Moreover, vanadium's biological function in bacteria has been characterised in some of the *Azotobacter* species, whereby vanadium replaces molybdenum in the FeMo-cofacter, which in turn may act as alternative nitrogenases (Slebodnick et al., 1997; Eady, 1996). Chromium is commonly used in industry which has led to large deposits of chromium into the environment. Only the mutagenic, carcinogenic and teratogenic hexavalent form of chromium (Cr(Vl)) and the less toxic trivalent chromium (Cr(lll) are of ecological importance as these compounds are in more stable oxidation states (Shen and Wang, 1995; Francisco et al., 2002). Reports have indicated the presence

of microbial groups that exhibit Cr(Vl) resistance and Cr(Vl)- reducing abilities including *Ochrobactrum anthropic* and *Acinetobacter Lwoffii* in activated sludge communities (Francisco et al., 2002). Sulphate reducing bacteria are a large group of diverse anaerobic bacteria that play a pivitol role in the cyclcing of carbon and sulphur in the environment (Rabus et al., 2006; Muyzer and Stams, 2008).

Significant positive correlations were observed between the total coliforms counts and the concentrations of magnesium present in the harvested rainwater samples. Douagui et al. (2012) and Nola et al. (2002) also observed significant positive correlations between coliform bacteria in groundwater and magnesium concentrations. Magnesium is the second most abundant element in cellular systems and is involved in basically all metabolic pathways for example, magnesium is an vital cofactor in almost all enzymes involved in DNA processing (for review see Hartwig, 2001).

Table 2.10. Major correlations between microbiological indicators and various metals and anions in rainwater samples ($p < 0.05$)

Variables	Spearman's r	p - value
V and Total Coliforms (SP*)	-0.407	0.02
Cr and Total Coliforms (SP*)	-0.370	0.04
Se and *E. coli* (MF*)	-0.437	0.01
Mg and Total Coliforms (SP*)	0.393	0.03
Si and Total Coliforms (SP*)	-0.415	0.02
SO_4 and Total Coliforms (SP*)	-0.415	0.02

2.4 Conclusions

The chemical quality of the rainwater, in the domestic rainwater harvesting tanks sampled in the Kleinmond Housing Scheme, were within the guidelines as stipulated by the Drinking Water Specification 241 of the South African National Standards (SANS, 2005), the South African Water Quality Guidelines for Domestic Water Use of the Department of Water Affairs and Forestry (DWAF, 1996) and the Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011). However, as the microbial counts obtained on average for all the indicator organisms, significantly exceeded ($p < 0.05$) the drinking water guidelines, harvested rainwater, that has been stored in polyethylene tanks for a short period of time (< 1 year), is not suitable for drinking purposes as per standards stipulated by the DWAF (1996) and the ADWG (NHMRC and NRMMC, 2011). Animals that have access to the catchment areas can be responsible for the presence of undesired bacteria. There was a lack of apparent areas (no trees) for birds and other animals to nest in, and this may have been an added advantage in lowering the risk for the contamination of the rainwater (Ahmed et al., 2011b; 2012a; b). However, as *E. coli* and faecal indicators were detected in this study, the faeces of birds, insects and mammals for example, could have filtered from the roof tops directly

into the rainwater tank which would have resulted in the faecal contamination of the rainwater. Rain allows pathogens from animal droppings and other organic debris to be flushed into the tanks via the gutters. A study performed in Southeast Queensland, Australia, demonstrated that identical biochemical phenotype profiles of *E. coli* strains were isolated from RWH tanks and from bird and possum faeces found on the roof surface. The results obtained thus suggested that the faeces of animals could have been the source of *E. coli* contamination in the RWH tanks (Ahmed et al., 2012b). As the total coliform counts were also above the standards in most samples, contamination could be as a result of particles, microorganisms, heavy metals and other organic substances as it is well known that these are some of the major pollutants found in the atmosphere that can potentially affect harvested rainwater. The poor microbial quality of the harvested rainwater could also potentially be assigned to dust. A gravel road runs along the outside of the settlement, and with cars passing by on a regular basis, dust could be disturbed and settle on the roof tops. The same observations were made for the rainwater in Hammanskraal in South Africa, where Nevondo and Cloete (1999) deemed the general quality of rainwater to be unacceptable. Other studies, world-wide, have also concluded that harvested rainwater is not suitable for drinking purposes without prior treatment (Yaziz et al., 1989; Sazakli et al., 2007; Zhu et al., 2004).

Prior treatment of the rainwater is therefore required before the water source can be utilised for drinking and certain domestic purposes. First flush diverters could be installed between the roof and the rainwater tank inlet to divert the first large amounts of debris which accumulates on the roof surfaces before rain events commence. This simple intervention could potentially significantly improve the microbial quality of the harvested rainwater. A study in Australia observed that diverting the first 2 - 5 mm of rain with the use of flush diverters improved the quality of the harvested rainwater by lowering the concentration of lead and organic matter (Kus et al., 2010). In addition, fluoride supplementation could potentially be included in the pre-treatment of harvested rainwater, if the rainwater is to be used as a primary drinking source (Sazakli et al., 2007). Further studies should be conducted to improve the microbial quality of harvested rainwater to within potable standards through the implementation of point of use treatment technologies, such as filter systems or solar disinfection.

2.5 References

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Chapter 3:

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Prevalence of virulence genes associated with pathogenic *Escherichia coli* strains isolated from domestically harvested rainwater during low and high rainfall periods

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ABSTRACT

The possible health risks associated with the consumption of harvested rainwater remains one of the major obstacles hampering its large scale implementation in water limited countries such as South Africa. Rainwater tank samples collected on eight occasions during the low and high rainfall periods (March to August 2012) in Kleinmond, South Africa were thus monitored for the presence of virulence genes associated with *Escherichia coli*. The identity of presumptive *E. coli* isolates in rainwater samples collected from ten DRWH tanks throughout the sampling period was confirmed through universal 16S rRNA PCR with subsequent sequencing and phylogenetic analysis. Species specific primers were also used to routinely screen for the virulent genes, *aggR, stx*, *eae* and *ipaH* found in Enteroaggregative *E. coli* (EAEC), Enterohaemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC) and Enteroinvasive *E. coli* (EIEC), respectively, in the rainwater samples. Of the 92 *E. coli* strains isolated from the rainwater using culture based techniques, 6% were presumptively positively identified as *E. coli* O157:H7 using 16S rRNA. Furthermore, virulent pathogenic *E. coli* genes were detected in 3% (EPEC and EHEC) and 16% (EAEC) of the 80 rainwater samples collected during the sampling period from the ten DRWH tanks. This study thus contributes valuable information to the limited data available on the on-going prevalence of virulent pathotypes of *E. coli* in harvested rainwater during a longitudinal study in a high population density, peri-urban setting.

Key Words: Domestic rainwater harvesting; *Escherichia coli*; *E. coli* virulent genes; longitudinal rainwater study

INTRODUCTION

Rainwater harvesting is practiced worldwide and refers to the collection and storage of rainwater runoff for domestic and agricultural use (1-3). Domestic rainwater harvesting (DRWH) can also potentially serve as a safe and inexpensive water supply for households and worldwide, countries such as Australia, Greece and Bermuda, are making extensive use of this water source (4-6). However, possible health risks associated with the consumption of harvested rainwater remains one of the major obstacles hampering the large scale implementation of DRWH, as microbial and chemical contaminants have previously been detected in rainwater tanks (7-9).

Depending on the atmospheric pollution, the harvesting method and the storage of rainwater, the quality of harvested rainwater may fluctuate and be compromised due to various pollutants, for example, bird or animal droppings (10). While generally an increase in the use of stored rainwater is observed during the high rainfall seasons, it should be noted that correspondingly increased levels of microbial pollution are also experienced during rainy seasons as large numbers of microorganisms are washed from various point- and non-point pollution sites, such as rooftops. Water then acts as an inert carrier of the pathogenic microorganisms, such as protozoa, helminths, viruses and bacteria, and humans can become infected with diseases such as diarrhea, skin irritations, typhoid and respiratory disorders from the microbially contaminated water sources (11). *Escherichia coli* (*E. coli*), a general indicator of water quality, can however also be pathogenic, and is divided into five classes namely Enteroaggregative *E. coli* (EAEC), Enterohaermorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC) and Enteroinvasive *E. coli* (EIEC), based on the specific virulent genes present. All the strains are associated with watery diarrhea but some strains are associated with bloody diarrhea (EHEC), vomiting (ETEC) and fever (ETEC and EIEC) (12).

The frequency of detecting *E. coli* strains during low and high rainfall seasons in DRWH tanks in Kleinmond, a coastal town in South Africa was monitored. The detection of the virulence genes associated with the four pathogenic *E. coli* strains (EAEC, EHEC, EPEC and EIEC) was particularly focused on. Spearman Rank Order Correlations were also determined between *E. coli*, rainfall, temperature, pH and between chemical compounds, for example, metal ions.

MATERIALS AND METHODS

Sample collection and general rainwater analysis

The Department of Science and Technology (DST) commissioned the Council of Science and Industrial Research (CSIR) to investigate technologies that will improve the sustainability and quality of low-income subsidized housing in South Africa (13). Consequently 411 pilot-scale houses (40 m^2) were constructed in Kleinmond, Western Cape, with each of the houses provided with a DRWH tank. From a cluster of 411 houses, 10 houses were selected for sampling rainwater during the study period (March to August 2012) and for the *E. coli* enumeration and identification. In addition, 29 houses (including the 10 for *E. coli* analysis) were selected for the statistical correlation studies. The vertical rainwater tanks, made of polyethylene, had a capacity of 2000 liters. There were no obstacles obstructing the roofs, i.e. trees or electrical power lines and no first flush diverters were installed to eliminate the first flush of debris from the roof surface into the tanks. Sampling was initially conducted every three weeks (March to May 2012) and there after one to four days after a rain event (June to August 2012).

For microbial and chemical analysis water samples were collected in 2 L sterile polypropylene bottles that were rinsed with tap water and sterilized with 70% ethanol. After collection the samples were stored on ice to maintain a temperature below 4°C during transportation. In total eight sampling sessions were conducted with data on the total rainfall recorded for each month obtained from the South African Weather Services (14).

Membrane filtration was also used to enumerate *E. coli* and the procedure was performed in duplicate within 4 hours of sampling. For sampling sessions one and two, undiluted samples were filtered. From sampling session three a 1:4 dilution was made of each sample in duplicate. The method consisted of filtering 100 mL (25 mL rainwater sample plus 75 mL sterile distilled water) of each sample through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The filters were then incubated on m-Endo Agar (Merck) at 35 ± 2°C for 18 - 24 hours (15). The membrane filtration results were utilized for the enumeration of *E. coli* and selection of isolates.

For each of the DRWH tanks an undiluted and diluted (10^{-1}) rainwater sample were spread plated onto Membrane Lactose Glucuronide Agar (MLGA) (Oxoid) (35 ± 2°C for 18 - 24 hours) to isolate *E. coli*. ChromoCult® Coliform Agar (CCA) (Merck, Biolab, Wadeville, Gauteng) was used to obtain *E. coli* numbers and the plates were also incubated at 35 ± 2°C for 18 - 24 hours. The

CCA counts were used for statistical purposes only, while isolates of *E. coli* were obtained from CCA and MLGA for the further selection of *E. coli*.

The temperature and pH of the rainwater at the sampling locations were measured using a hand-held mercury thermometer and color-fixed indicator sticks with a pH range of 0 - 14 (ALBET®, Barcelona, Spain). The concentrations of metals such as aluminum (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn), amongst others, were determined for the first sampling. Metal concentrations were determined using nitric acid digestion and inductively coupled plasma atomic emission spectrometry (ICP-AES). All chemical analyses were performed at the Central Analytical Facility (CAF), Stellenbosch University. High-Performance Ion Chromatography (HPIC) was used to determine the concentration of anions such as, chloride (Cl), nitrate (NO_3) and sulphate (SO_4) during the first sampling.

Molecular Analysis of *Escherichia coli*

Isolation of *Escherichia coli* and Genomic DNA Extractions

Typical *E. coli* isolates from 10 randomly selected rainwater tanks (within the cluster of 29 tanks monitored) were selected from ChromoCult® Coliform Agar, m-Endo agar and MLGA agar, which specifically selects for the growth of *E. coli* and suppresses the growth of other enteric species. These isolates were then subjected to the IMViC test for the further selection of *E. coli* strains (16).

After purification and IMViC test analysis, single colonies obtained from nutrient agar were inoculated into Luria Bertani (LB) broth (Merck) and were grown at 37°C for 12 h. Cells were harvested from 2 mL of the cell suspension by centrifuging at 16 000 × g for 10 min and discarding the supernatant. Genomic DNA was then isolated according to the manufacturer's instructions using the ZR^{TM} Soil microbe DNA Miniprep Kit (Zymo Research).

Universal 16S rRNA identification of *E. coli* isolates

With the use of conventional PCR, presumptive positive *E. coli* isolated from rainwater samples were identified. Universal 16S rRNA primers Fdd2 (5'-CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG- 3') and Rpp2 (5' - CCAAGCTTCTAGACGGITACCTTGTTACGACTT - 3') and the PCR conditions adapted from Rawlings (17), were used to amplify the 16S rRNA conserved region (1600 bp) of each isolate. The PCR conditions were optimized by increasing the annealing temperature from 54°C to 59°C.

Phylogenetic trees of the results obtained for the 16S rRNA *E. coli* sequences were constructed to observe whether non-pathogenic and pathogenic strains of *E. coli* clustered together

and how this varied between certain sampling sessions (18). Most similar type species with 97% similarity (< 3% diversity) to the sequences of isolates were designated as the same species. The 16S rRNA sequences were aligned using Clustal X (19, 20). The evolutionary distances for each 16S rRNA were also calculated by the neighbor-joining method with Maximum Composite Likelihood model by 1000 replicates and phylogenetic trees were created by using MEGA (21). All positions containing gaps and missing data were eliminated from the dataset using complete deletion option.

Screening for pathogenic *E. coli* genes in rainwater samples

In addition to extracting DNA from each presumptive *E. coli* isolate, total DNA was extracted from rainwater samples collected from ten tanks. To extract total DNA from the water samples a modified version of the boiling method proposed by Watterworth et al. (22) was used. Eight hundred milliliters of each sample was filtered through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences) with a pore size of 0.45 µm and a diameter of 47 mm. The filters were then processed for DNA extraction as outlined in Ndlovu et al. (23).

The species specific primers adopted from Toma et al. (24) that were used for the detection of the pathogenic *E. coli* strains (EPEC, EIEC, EHEC and EAEC) in the rainwater samples are indicated in Table 3.1.

TABLE 3.1. Primer sequences used in this study for the identification and detection of *E. coli* (24)

Bacteria	Primer set 5'-3'	Primer Name	Target gene	Product size
EAEC	GTATACACAAAAGAAGGAAGC	AggRKs1	*aggR*	254 bp
	ACAGAATCGTCAGCATCAGC	AggRkas2		
EHEC	GAGCGAAATAATTTATATGTG	VTcomU	*stx*	518 bp
	TGATGATGGCAATTCAGTAT	Vtcomd		
EPEC	CCCGAATTCGGCACAAGCATAAGC	SK1	*eae*	881 bp
	CCCGGATCCGTCTCGCCAGTATTCG	SK2		
EIEC	GTTCCTTGACCGCCTTTCCGATACCGTC	IpaIII	*ipaH*	619 bp
	GCCGGTCAGCCACCCTCTGAGAGTAC	IpaIV		

All the positive control strains (EPEC B170, EIEC ATCC 43892, EHEC O157:H7 and EAEC 3591-87) utilized in this study were obtained from the Cape Peninsula University of Technology (CPUT), Bellville. The PCR conditions and reagents utilized were adapted from the Ndlovu et al. (23) protocol as outlined in Table 3.2. The PCR conditions were also optimized by increasing the annealing temperature from 52°C to 54°C.

TABLE 3.2. PCR condition and reagents used to detect pathogenic *E. coli* in DNA extracted from water samples (23)

PCR Reagent	EAEC (µl)	EHEC (µl)	EPEC (µl)	EIEC (µl)	Final concentration	PCR conditions
Buffer (5X)	12	12	12	12	1X	Stage 1: Initial denaturing at 95°C for 2 min Stage 2: Denaturing at 95°C for 1 min, primer annealing at 54°C for 1 min and elongation at 72°C for 1 min Stage 3: Final elongation step at 72°C for 10 min.
$MgCl_2$ (25 mM)	6	6	6	6	2.5 mM	
AggRKs1 (10 µM)	1				0.16 µM	
AggRkas2(10 µM)	1					
VTcomU (10 µM)		1.5			0.25 µM	
Vtcomd (10 µM)		1.5				
SK1 (10 µM)			0.75		0.125 µM	
SK2 (10 µM)			0.75			
IpaIII (10 µM)				0.6	0.1 µM	
IpaIV (10 µM)				0.6		
dNTP (10 mM)	1.2	1.2	1.2	1.2	0.2 mM	
GoTaq Polymerase (5 U/µl)	0.5	0.5	0.5	0.5	2.5 U	
Template DNA	5	5	5	5	5	
dH_2O	33.3	32.3	33.8	34.1		
Final volume	60	60	60	60	60	

Statistical Analysis

The data obtained from the microbial and physico-chemical analysis of the collected rainwater samples was assessed using the statistical software package Statistica™ Ver. 11.0 (Stat Soft Inc, Tulsa, USA). In each data set, analysis of the residuals revealed that the data was not normally distributed, which pointed to the requirement for the Spearman Rank Order Correlation as non-parametric correlation technique to test the significance of the data set. In this test, a Restricted Maximum Likelihood solution (REML) with type III decomposition was performed on all data recorded to establish whether there was variation between sampling sessions. Once it was established that variation was indeed present, Variance Estimation, Precision and Comparison (VEPACK) analysis was performed. However the data for pH, temperature and average rainfall were set as fixed variables and time and sample were set as grouping variables. Data pairs that showed significant differences were subsequently further analyzed using the Least Squares Difference (LSD) test and probabilities for Post-hoc pair-wise comparisons. In all hypothesis tests, a significant level of 5% was used as standards (25). In all tests a P-value smaller than 0.05 was considered as statistically significant.

RESULTS

Prevalence of *Escherichia coli* during low and high rainfall period

On average the membrane filtration *E. coli* counts for the ten DRWH tanks ranged from zero (for isolated tanks) to 2.5×10^2 cfu 100 mL^{-1} over the entire sampling period. Throughout sampling one to four, the low rainfall period (16.8 mm in March to 30.6 mm in May), numerous tanks had no *E. coli* present with 44% of the DRWH tanks sampled exceeding the drinking water guidelines as stipulated by the Department of Water Affairs (DWAF) (26) and the Australian Drinking Water Guidelines (ADWG) (27). In comparison, for sampling five to eight, where higher rainfall events (74.7 mm in June to 198.1 mm in August) were recorded, 79% of the tanks sampled had *E. coli* counts significantly ($p < 0.05$) exceeding the standards. Overall for all the rainwater samples collected from the domestic rainwater harvesting tanks (1 to 29) for sampling one to eight, utilizing membrane filtration, 62% of the *E. coli* counts recorded exceeded the recommended values as stipulated by DWAF (26) and the ADWG (27).

Identification of *E. coli* isolates based on 16S rRNA analysis

Of the 170 presumptive positive *E. coli* plate isolates identified throughout the sampling period from ten DRWH tanks, 71% (121 strains) yielded a positive IMViC analysis and exhibited *E. coli* characteristics. The identity of these presumptive *E. coli* strains was then confirmed through universal 16S rRNA PCR with subsequent sequencing. Sequencing revealed that 76% (92 strains) of the IMViC positive isolates were *E. coli* strains, while the remaining isolates belonged to the genera *Enterobacter, Serratia, Shigella* and *Proteus*.

The phylogeny of the representative organisms according to GenBank for samplings 1, 3 and 6 (greater *E. coli* strain diversity identified during these sampling times) were analyzed using the neighbor-joining algorithm in ClustalX (phylogenetic tree for sampling 3 only presented). Among the 92 *E. coli* isolates that were identified using GenBank, 4% were positively identified as the enterotoxigenic *E. coli* (ETEC), which contains the heat stable toxin (ST1). These presumptive positive ETEC isolates were identified predominantly in sampling 1 and 3. In addition 6% of the total *E. coli* isolates were identified as *E. coli* O157:H7 (sampling 1, 2 and 3).

Three ETEC strains were identified during sampling 1 (results not shown), with two of the strains clustering together with a 99% statistical support. The *E. coli* strains most frequently isolated and identified during sampling 2 (results not shown) and 3 (Fig. 3.1) were the *E. coli* O157:H7 strains WAB1892 and TW14359, respectively, and *E. coli* 0111:H- strain 11128 (sampling 3).

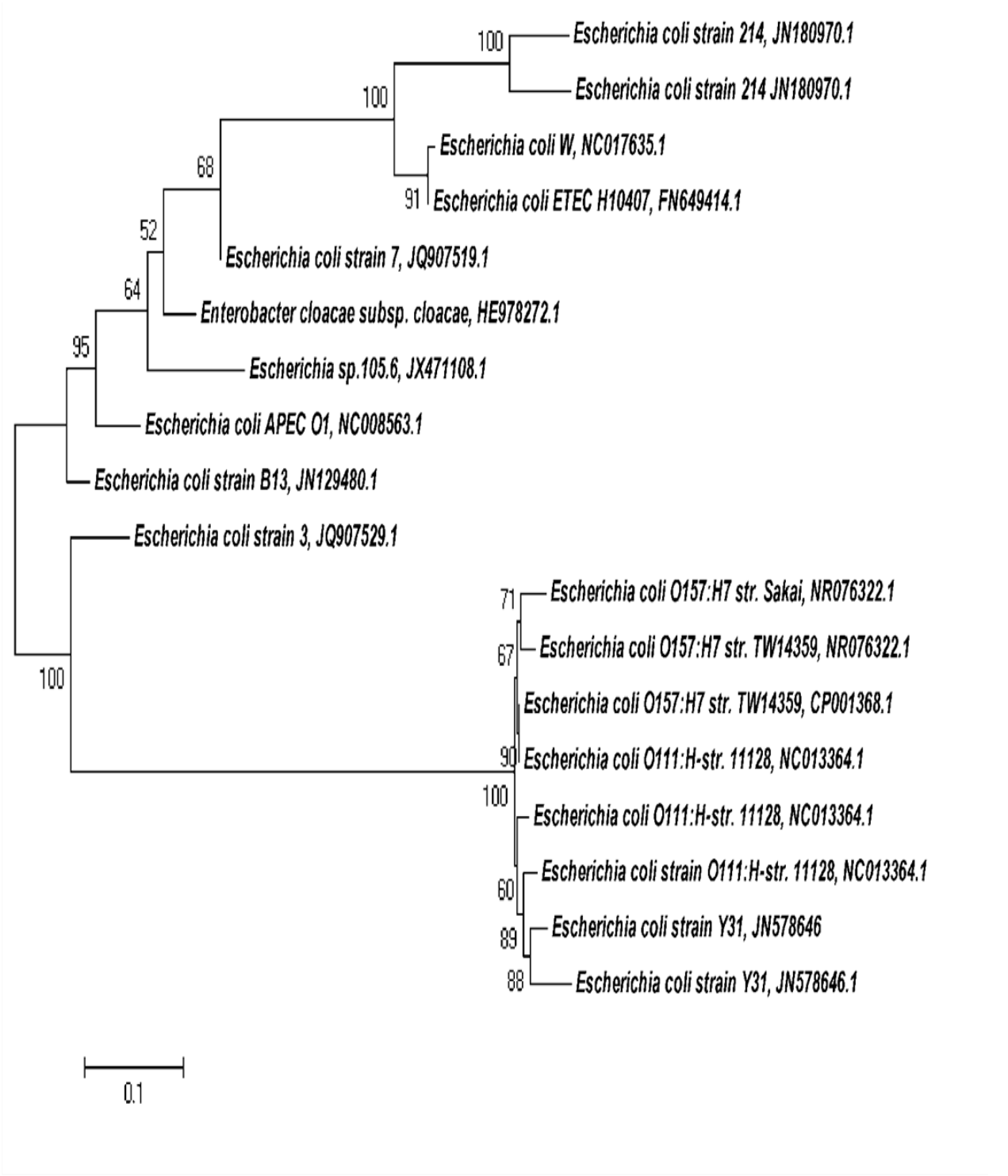

FIG 3.1. An unrooted phylogenetic tree of organisms isolated during sampling three. The tree of isolates was constructed using the neighbor-joining algorithm of ClustalX. Bootstrap values are shown at the nodes with the accession numbers indicated after the strain name.

From Fig. 3.1 it is clear that these two strains, namely *E. coli* O157:H7 strain TW14359 and *E. coli* 0111:H- strain 11128 cluster together. *Escherichia coli* 0111:H- and O157:H7 are both main serotypes that produce Shiga toxins which could explain their homology (28). *Escherichia coli* 0111:H- was also isolated during sampling 6, 7 and 8 (results not shown). From the 16S rRNA PCR analysis, the majority of the pathogenic strains of *E. coli* were isolated during sampling 1, 2 and 3, where the temperatures were higher and the total rainfall was low (16.8 mm in March to 30.6 mm in May). In addition, an avian isolate *E. coli* APECO1 was identified during the third sampling period. The dominant strain identified in sampling 6 was *E. coli* DSM 1103 (data not shown). Results indicated that the two genera, *Shigella* and *Escherichia* also grouped together and this could be attributed to the fact that they are closely genetically related (29).

Presence of pathogenic *E. coli* genes in rainwater samples

Species specific primers were used to screen for the virulent genes, *aggR, stx, eae* and *ipaH* found in Enteroaggregative *E. coli* (EAEC), Enterohaemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC) and Enteroinvasive *E. coli* (EIEC), respectively, in the rainwater samples collected from the ten DRWH tanks. These PCR products were sequenced to confirm the amplification of the gene and BLAST results that showed a homology of $\geq$ 98% was confirmed as the amplification of the correct gene. The virulent genes that were amplified in the various tanks throughout the sampling period are presented in Tables 3.1 and 3.3, and as indicated no pathogenic *E. coli* genes were detected during sampling sessions one, two, three and five.

In this study, the *aggR* gene, associated with the EAEC strain, was detected in 10% of the rainwater tanks during samplings 4 and 6, respectively (Table 3.3). Detection of the *aggR* gene was also confirmed in 50% and 60% of the DRWH tanks during sampling sessions 7 and 8. The occurrence of EPEC [intimin gene (*eae*)] and EHEC [shiga toxin gene (*stx*)] was much lower than EAEC throughout the sampling period, but was confirmed in sampling 6 and 8 (Table 3.3) at 10% for EPEC (sampling 6) and at 20% for EHEC (sampling 8).

TABLE 3.3. The presence of *Escherichia coli* harbouring toxin genes in rainwater samples

Sampling number	Percentage rainwater tanks contaminated with *E. coli* toxin genes	*EPEC *eae*	*EIEC *ipaH*	*EHEC *stx*	*EAEC *aggR*
4	10%	ND	ND	ND	9
6	20%	5	ND	ND	4
7	50%	ND	ND	ND	2, 6, 8, 7, 10
8	90%	1	ND	9, 10	1, 2, 3, 5, 6, 10

ND-Not detected

* Indicates tanks where genes were isolated.

Spearman Rank Order Correlations between *E. coli* and physico-chemical properties of rainwater

For the statistical correlation studies, the results for 29 houses (including the 10 for *E. coli* analysis) were analysed. Significant correlations ($p < 0.00$) were noted between *E. coli* counts, utilizing the spread plate technique (CCA) and the following parameters: *E. coli* counts utilizing membrane filtration ($R = -0.21$) (m-Endo) and rainfall ($R = -0.36$). It should also be noted that in the current study the pH ($R = -0.655$) and temperature ($R = -0.705$) of the rainwater samples showed a significant negative correlation to the average rainfall recorded. After analyzing all the data the REML and the Fixed Effect test showed significant variation ($p = 0.00$, $F = 8.968$) for *E. coli* counts (utilizing the spread plate technique) amongst the eight sampling sessions. The LSD test then showed that the highest mean difference (2465.52±449.86) ($p = 0.00$) was recorded between sampling sessions one and seven, while the lowest mean difference, with the least amount of variation, was recorded between sampling sessions two and six (68.97± 449.8644) ($p = 0.878$).

DISCUSSION AND CONCLUSIONS

Escherichia coli is included as a specific indicator organism of the level of fecal pollution from warm-blooded animals (4, 30) and the presence of *E. coli* in harvested rainwater samples was thus monitored in the low and high rainfall seasons in DRWH tanks in Kleinmond, South Africa. Enumeration techniques indicated that the *E. coli* counts recorded exceeded the recommended value of 0 cfu 100 mL^{-1} (26, 27) in 62% of the rainwater samples collected throughout the study period. In comparison to the low rainfall season, a significant increase ($p < 0.05$) in the numbers of *E. coli* were also observed in harvested rainwater samples collected during the high rainfall season [sampling five (74.7 mm) to eight (198.1 mm)]. The percentages of *E. coli* detected in similar

studies conducted on the microbial quality of rainwater also varied from 33% (9), 63% (31) to 79% of the samples (32). However, these studies were performed 1 to 4 days after a rain event and, to our knowledge, only the study performed by Sazakli et al. (4) investigated the effect of seasonal variation on the quality of harvested rainwater, with the highest *E. coli* numbers recorded in autumn, which is the start of the rainfall season. A negative correlation was also recorded between pH and temperature and rainfall, which implies that *E. coli* numbers increased during sampling five to eight when the rainfall was high and the pH [sampling five (pH 5.4) to eight (pH 5.1)] and temperature [sampling five (14.8 °C) to eight (13.3 °C)] were low.

Ninety-two *E. coli* strains were subsequently isolated by culture techniques from the 10 rainwater tanks sampled in the Kleinmond Housing Scheme during the study period with a preliminary identification performed using 16S rRNA analyses. Non-pathogenic and pathogenic *E. coli* strains, including *E. coli* ETEC H10407, *E. coli* O157:H7 and an avian isolate *E. coli* APECO1, were isolated from numerous DRWH samples during the study. The ETEC strain identified predominantly in sampling 1 and 3, causes watery diarrhea and in a few cases it can also cause vomiting and fever. Infants and travelers in underdeveloped countries are the most susceptible to this *E. coli* strain (33). *Escherichia coli* O157:H7, isolated during sampling 2 and 3, is the predominant serotype of enterohaemorrhagic *E. coli* (EHEC) that produces the Shiga-like toxin that is responsible for causing watery and subsequent bloody diarrhea in humans. In addition, the avian isolate *E. coli* APECO1, isolated during sampling 3, most likely originated from bird feces and may contain many virulent genes belonging to the extra-intestinal pathogenic *E. coli* (ExPEC).

The whole DNA isolated from the harvested rainwater samples directly was then screened for the presence of the virulence genes, *aggR, stx, eae* and *ipaH* associated with Enteroaggregative *E. coli* (EAEC), Enterohaemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC) and Enteroinvasive *E. coli* (EIEC), respectively. The most prevalent gene detected during the study was *aggR*, associated with EAEC strains. This virulence gene was detected during sampling 4, 6, 7 and 8, at 10%, 10%, 50% and 60%, respectively. The presence of EAEC harboring the *aggR* gene in these tanks can have a severe impact on the health of children and adults. In addition, while the occurrence of EPEC [intimin gene (*eae*)] and EHEC [shiga toxin gene (*stx*)] was much lower than EAEC throughout the sampling period, the presence of these genes were also confirmed in sampling 6 and 8 (Table 3.3) at 10%, respectively for EPEC and at 20% for EHEC (sampling 8). The detection of the intimin *eae* gene in EPEC could indicate the presence of shiga-toxin producing *E. coli* (STEC), since this gene is found in more than one pathotype (34). The ingestion of EPEC however, causes watery diarrhea that is associated with vomiting and low fever (12), while the EHEC is one of the most pathogenic *E. coli* groups that have the ability to cause bloody diarrhea,

with little or no fever. If the disease is left untreated, it can lead to haemorrhagic colitis (HC) and can progress to haemolytic-uraemic syndrome (HUS), affecting the kidney and liver. The virulence gene *ipaH*, associated with EIEC strains, was not detected in any of the rainwater samples collected during the sampling period.

It is important to note that during sampling 1, 2, 3 and 5 no virulence genes were detected in the rainwater tanks. Ten per cent of the rainwater samples collected from the tanks during sampling 4 were contaminated with virulence genes, while sampling 8 had the highest number of tanks (90%) contaminated. Overall, during sampling 4, 6, 7 and 8, 10%, 20%, 50% and 90%, respectively of the tanks were contaminated with pathogenic *E. coli* toxin genes. It is also evident that the virulent genes were most often detected during sampling 8 where the highest average rainfall of 198 mm was recorded. On comparison, these results are contradictory to the 16S rRNA analysis where pathogenic *E. coli* (O157:H7 and ETEC) were most frequently identified from culturable *E. coli* strains during sampling 1 to 3. It can thus clearly be seen that 16S rRNA may not be not suitable to distinguish between strains in a species, as was also confirmed by Lukjancenko et al. (29). Results of this study also clearly indicate that the *E. coli* plate counts and the presence of *E. coli* virulence genes were lower in the low rainfall season (sampling 1 to 4) and significantly increased ($p < 0.05$) in the high rainfall season (sampling 5 to 8). The feces of warm-blooded animals could serve as a possible source of *E. coli* contamination in the rainwater tanks, as warm blooded animals have been shown to carry a high number of pathogenic *E. coli* strains in their intestines (36). Ahmed et al. (35) conducted a study in Southeast Queensland, Australia, and successfully isolated *E. coli* species with identical biochemical phenotype profiles from rainwater tanks as well as from bird and possum feces found on the roof surfaces where the tanks were installed. Other sources of rainwater contamination include “leaf debris and organic material washed into the tank, animals, insects and birds that have drowned in the water and breeding mosquitoes” (37). A recent study conducted in Singapore confirmed the presence of pathogenic microorganisms, including *E. coli*, in airborne particulate matter which can serve as another source of contamination (38). A gravel road also runs along the outside of the settlement, and with cars passing by on a regular basis, dust could be disturbed and settle on the roof surface, resulting in contamination.

Although studied serotypes of *E. coli* are well recognized as being of zoonotic origin these *E. coli* strains have been shown to infect humans and are therefore a health risk if present in a water source. For example EHEC strains are known to be of zoonotic origin with animals such as cattle being the reservoir for human infections (39). Therefore, even though microbial source tracking with the use of sewage associated markers such as *Bacteroides* HF183 (40), could be used to determine if fecal contamination is of human origin, the focus of this study was on the detection of

pathogenic *E. coli* serotypes present in harvested rainwater which could have potential health risks. Additional studies have also been conducted which focused on the detection of bacterial pathogens associated with human diseases, such as *Salmonella* and *Shigella* spp. in the harvested rainwater collected from DRWH in Kleinmond, South Africa.

In conclusion, the *E. coli* counts, isolation and identification of *E. coli* strains and the detection of virulence genes associated with EAEC, EHEC and EPEC strains in the rainwater samples, clearly indicates that the roof harvested rainwater is not suitable for potable purposes, with limited domestic application, as this water source could be associated with public health risks and human disease. Similar observations were made world-wide, where it was concluded that harvested rainwater is not suitable for drinking purposes without prior treatment (4, 7, 8, 41). In addition, rain allows pathogens from animal droppings and other organic debris to be flushed into the tanks via the gutters and as *E .coli* counts and toxin genes were increased during the higher rainfall period, the feces of birds, insects and mammals, could have filtered from the roof tops into the rainwater tank, which would have resulted in the fecal contamination of the water source. However, harvested rainwater is an important, alternative water source that could be utilized if the technology is applied in the correct manner and if the rainwater is treated before it is used for drinking and certain domestic purposes. Therefore promoting the correct use and maintenance of DRWH tanks could improve the microbial and chemical quality of the harvested rainwater (42, 43). Future research is thus focusing on the implementation of point of use systems, such as nanofiltration, solar pasteurization, etc. for the treatment of harvested rainwater sources. In addition, currently solar pasteurization and filtration systems are being optimized and analyzed at the pilot plant scale level and the efficiency and durability of these systems in improving the microbial quality of harvested rainwater are being investigated.

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Distribution of indigenous bacterial and potential pathogens associated with roof-harvested rainwater

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ABSTRACT

The harvesting of rainwater is gaining acceptance amongst many governmental authorities, in countries such as Australia, Germany and South Africa, amongst others. However, conflicting reports on the microbial quality of harvested rainwater have been published. To monitor the presence of potential pathogenic bacteria, during high rainfall periods, rainwater from 29 rainwater tanks were sampled on four occasions (June and August 2012) in a sustainable housing scheme in Kleinmond, South Africa. This resulted in the collection of 116 harvested rainwater samples in total throughout the sampling period. The identity of the dominant, indigenous, presumptive pathogenic isolates obtained from the rainwater samples throughout the sampling period were confirmed through universal 16S rRNA PCR, with the results revealing that *Pseudomonas* (19%) was the dominant genus isolated, followed by *Aeromonas* (16%), *Klebsiella* (11%) and *Enterobacter* (9%). Polymerase chain reaction assays with the use of genus specific primers also confirmed the presence of *Aeromona*s spp. (16%), *Klebsiella* spp. (47%), *Legionella* spp. (73%), *Pseudomonas* spp. (13%), *Salmonella* spp. (6%), *Shigella* spp. (27%), and *Yersinia* spp. (28%) in the harvested rainwater samples. In addition, on one sampling occasion, *Giardia* spp. were detected in 25% of the eight tank water samples analyzed. This study highlights the diverse array of pathogenic bacteria that persist in harvested rainwater during high rainfall periods. The consumption of untreated harvested rainwater could thus pose a potential significant health threat to consumers, especially children and immunocompromised individuals, and it is recommended that harvested rainwater be treated for safe usage as an alternative water source.

Key Words: Rainwater harvesting; indigenous bacterial pathogens; *Giardia* spp.

INTRODUCTION

Rainwater harvesting (RWH) has been described as an alternative improved water source as this technology could assist in the provision of water directly to households for drinking and domestic purposes (1). Communities are also able to capture and store rainwater for utilization in small-scale productive activities, such as vegetable gardening, which could make a positive contribution towards food security for individuals from lower socioeconomic groups (2). In addition, low economic growth and the effects of climate change have compelled many governments and water authorities worldwide to rely on the process of harvesting rainwater as an alternative source of water (3).

A limited number of qualitative studies (4) have been conducted on the usage of rainwater for domestic and potable purposes, and while some studies have determined that harvested rainwater is safe for drinking purposes without prior treatment (5, 6), a few studies have however, shown that harvested rainwater is, in fact, not suitable for potable purposes (7, 8, 9, 10, 11). As rainwater is collected from roof surfaces, pathogenic organisms that are found in bird feces, insects, mammals, reptiles and other debris, may be flushed into the tanks via the gutters and the tank inlet systems. This could pose serious human health risks (12) and between 1978 and 2006, six incidences of disease related to rainwater were reported (10, 13, 14).

Indicator organisms, such as fecal coliforms and *Escherichia coli*, are routinely used to monitor the microbial quality of water sources (6, 15, 16). However, studies have shown that there is a poor correlation between indicator organisms and pathogenic organisms in environmental water samples (17, 18, 19) and for this reason Ahmed et al. (9) questioned whether the detection of indicator organisms was sufficient in determining the quality of rainwater. This discrepancy is attributed to the fact that firstly, indicator organisms have been shown to have different survival rates compared to pathogens and secondly, fecal indicators may replicate in external environments (20, 21). Polymerase Chain Reactions (PCR) based techniques have also been used in numerous studies to identify and quantify bacterial pathogens in environmental waters as this technique offers a rapid, precise and sensitive identification of bacterial pathogens. For example Ahmed et al. (15) used PCR in the detection of *Bacteroides* spp. and detected the fecal indicator in 89% of the rainwater samples, while Savill et al. (22) detected *Campylobacter* spp. with the use of PCR based methods in 37.5% of the roof-collected water samples. Enteric pathogens such as *A. hydrophila, C. jejuni, C. coli* and *Salmonella* spp. were also detected in harvested rainwater through PCR and were present in 7%, 19%, 1% and 17% of the samples, respectively (9). Protozoa pathogens detected in rainwater included *Giardia* spp. and *Cryptosporidium* spp. (23, 24).

As South Africa is currently implementing strategies to utilize harvested rainwater as an alternative water source, the aims of this study were (i) to identify the dominant indigenous bacterial isolates cultured from harvested rainwater samples during a high rainfall season by using 16S rRNA sequencing and (ii) to investigate the presence and the frequency distribution of pathogenic bacteria that are ubiquitous in harvested rainwater samples with the use of genus specific PCR detection.

MATERIALS AND METHODS

Sample site and collection.

Sampling was conducted at a sustainable, low-income, subsidized housing project in Kleinmond, a coastal town situated in the Western Cape, South Africa (25). Twenty-nine houses with vertical, polyethylene domestic rainwater harvesting tanks (capacity of 2000 L) installed, were selected from a cluster of 411 houses established by the Council for Scientific and Industrial Research (CSIR) and the Department of Science and Technology in 2010. No first flush diverters were installed above the tanks to eliminate the first flush of debris from the roof surface into the tanks. Four sampling sessions were conducted from June to August 2012, with sampling being conducted one to four days after a rain event. Rainfall data for the Kleinmond area was obtained from the South African Weather Services (Pretoria, South Africa). On each of the four sampling occasions, 2 L rainwater samples were collected from each of the 29 domestic rainwater harvesting tanks, resulting in the total collection of 116 samples. Additional 5 L rainwater samples were collected from eight domestic rainwater harvesting tanks for the detection of G. lamblia and C. parvum in one sampling occasion (third sampling session). After collection the samples were stored on ice to maintain a temperature below 4°C during transportation.

Culturing and isolation of the dominant indigenous bacteria from harvested rainwater samples.

In order to isolate Gram-negative enteric microorganisms from each rainwater tank sample collected during the four sampling sessions, 1 mL of the original sample was used in a serial dilution. Thereafter 100 µL of an undiluted and diluted (10-1) sample (processed within 36 hours) was spread plated onto salmonella - shigella (ss) agar (Merck, Biolab, Wadeville, South Africa), and incubated at 35 ± 2°C for 18–24 hours. The undiluted and diluted tank water samples were also spread plated onto legionella charcoal-yeast extract (CYE) agar base (Oxoid, Hampshire, England), supplemented with glycine, vancomycin, polymyxin B and cycloheximide (GPVC) vials SR0152 and SR0110 (Oxoid), and incubated at 35°C for 48 hours in order to isolate Legionella spp. The selective detection was then increased by pre-incubating the agar plates at 50°C for 30 min before cultivation (26). In order to isolate Campylobacter species, an undiluted and a diluted (10-1) tank

water sample was spread plated onto Campylobacter blood-free selective medium (Oxoid) with the selective supplement SR0155 (Oxoid) added according to the manufacturer's instructions. Plates were incubated under microaerophilic conditions with the use of an Anaeropack® Anaero (Davies Diagnostics, Randburg, South Africa) in a sealed container. This container was incubated at 35°C for approximately 10 days.

Isolates were categorized based on morphological similarities and representatives from each category were identified with the use of molecular techniques to identify the dominant, indigenous microorganisms that were able to grow on ss agar, legionella GPVC agar and on campylobacter selective media under micro-aerobic conditions.

Genomic DNA extractions from bacterial isolates and tank water samples.

The selected isolates were sub-cultured onto nutrient agar (NA) (Merck, Biolab, Wadeville, South Africa) at least three times and once pure colonies had been obtained a single colony was inoculated into 5 mL Nutrient Broth (Merck) and incubated at 37°C overnight. Cells were harvested by centrifuging 2 mL of each culture for 10 min at 6000 × g. The extraction of genomic DNA from each isolate was then performed using a soil microbe DNA miniprep kit (Zymo Research) according to the manufacturer's instructions.

For the PCR analysis of potentially pathogenic bacteria, total DNA extractions were also performed for each of the 116 tank water samples collected during sampling session one to four. In order to extract total genomic DNA from the tank water samples a modified version of the boiling method was utilized (27). Each tank water sample (1.6 L) was filtered through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm at a flow rate of approximately ≥ 65 mL/min/cm^2 at 70 kPa. In the incidences where the filters were saturated, more than one filter was used to filter the 1.6 L volume of tank water. The filters were then processed for DNA extraction as outlined in Ndlovu et al. (28). Briefly, the filters for each rainwater sample were incubated in 2 mL Luria Bertani (LB) (Merck, Biolab, Wadeville, South Africa) broth for 5 hours at 37°C. The samples were vortexed for 15 min to detach the cells from the filters and cells were harvested from 2 mL of the cell suspension through centrifugation at 12500 × g for 10 min. The supernatant was discarded and the pellet re-suspended in 100 µL sterile MilliQ water. The re-suspended cells were then boiled at 95°C for 15 min, followed by cooling on ice for 10 min. The sample was subjected to centrifugation at 12500 × g for 5 min and the supernatant transferred to a sterile eppendorf tube. Genomic DNA and total DNA was then visualised on a 0.8% agarose gel stained with 0.5 µg/mL ethidium bromide.

Universal 16S rRNA identification and phylogenetic analysis of dominant bacterial isolates.

Once genomic DNA had been extracted from the various isolates, polymerase chain reactions (PCR) were used to amplify the 16S rRNA conserved sequence. The PCR mixture consisted of a final volume of 50 µL and contained 10 µL of 5X Green GoTaq® Flexi Buffer (1X final concentration; Promega) (1X), 4 µL $MgCl_2$ (2.0 mM), 0.5 µL of each dNTP (0.1 mM) (Thermo Scientific, Lithuania), 2.5 µL of each PCR primer (0.5 µM) (Table 4.1), and 0.3 µL (1.5 U) of GoTaq® Flexi DNA Polymerase (Promega, Madison, USA). Amplification was performed in a Thermal Cycler (Bio-Rad, USA) as outlined in Table 4.1.

Phylogenetic trees of the results obtained for the 16S rRNA sequences were analyzed as outlined in Jackson et al. (39). The sequences of representative isolates, that showed > 97% similarity (< 3% diversity) to organisms recorded on the international databases, such as Genbank, were used in the construction of the phylogenetic trees. The 16S rRNA sequences were aligned using the default settings and BLOSUM matrix (for the correction of multiple base changes) of Clustal X (1.81) (40). To calculate the distances of relatedness between each sequence, unrooted trees were assembled using the neighbor-joining method and Maximum Composite Likelihood function (41). Phylogenetic analysis according to Tamura et al. (42) was done using the program Molecular Evolutionary Genetics Analysis Version 3.1 (MEGA, version 3.1) where bootstrap values were set at 1000. Positions that contained missing data were eliminated from the dataset using the complete deletion option.

Genus-specific PCRs from tank water samples.

Primers and PCR conditions as outlined in Table 4.1 were utilized in the current study for the identification of pathogenic and opportunistic bacterial pathogens that have previously been identified in rainwater tank samples. Each PCR mixture was performed in a final volume of 50 µL. For the detection of *Shigella* spp., *Salmonella* spp. and *Aeromonas* spp. the PCR mix consisted of 10 µl of 5X Green GoTaq® Flexi Buffer (Promega) (1X), 4 µL MgCl2 (2.0 mM) (Promega), 0.5 µl of each dNTP (0.1 mM) (Thermo Scientific), 0.5 µL of the PCR primer (0.1 µM), 0.3 µL of GoTaq® Flexi DNA Polymerase (Promega) (1.5U) and 10 µL of template DNA. For *Campylobacter* spp., *Yersinia* spp. and *Klebsiella* spp. the same PCR mix was used with the exception that 1.5-µL of the respective forward and reverse PCR primers (0.3 µM) was added. For *Pseudomonas* spp. and *Legionella* spp., again, the same reaction mixture was used with 10 µL of template DNA; however 2.0 and 2.5 µL of each PCR primer (0.4 and 0.5 µM, respectively) was also used, respectively.

For each PCR reaction a negative control, consisting of sterile water, and a positive DNA control were included. The following strains were cultured as positive controls after which, genomic DNA was extracted; *Legionella pneumophila* ATCC 33152, *Shigella sonnei* ATCC 25931, *Salmonella tymphimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Campylobacter jejuni* subsp. *jejuni* ATCC 33291, *Aeromonas hydrophila* (environmental strain), *Klebsiella pneumoniae* ATCC 13385 and *Yersinia enterocolitica* ATCC 27729. Unless indicated otherwise, all positive control organisms were obtained from Microbiologics®. The specificity of each primer set was confirmed by using non target DNA extracted from all the above mentioned positive controls.

TABLE 4.1**:** Primers and PCR cycling parameters for the detection of various potential bacterial pathogens

Organism	Primer name	Primer sequence (5'-3')	PCR Cycling Parameters	Gene (Size bp)	References
Universal	fDD2	CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG	3 min at 94°C; 30 cycles of 94°C for 30 s, 53°C for 30 s min, 72°C for 1.5 min	16S rRNA (1600)	29
	rPP2	CCAAGCTTCTAGACGGITACCTTGTTACGACTT			
Legionella spp.	JFP	AGGGTTGATAGGTTAAGAGC	5 min at 95°C; 40 cycles of 94°C for 1 min, 57°C for 1.5 min, 72°C for 1 min	Attachment invasion locus gene (386)	30
	JRP	CCAACAGCTAGTTGACATCG			
Aeromonas spp.	Aero-F	TGTCGGSGATGACATGGAYGTG	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Aerolysin (720)	31
	Aero-R	CCAGTTCCAGTCCCACCACTTCA			
Shigella spp.	IpaH-F	CCTTGACCGCCTTTCCGATA	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Invasion plasmid Antigen H (606)	31
	IpaH-R	CAGCCACCCTCTGAGGTACT			
Salmonella spp.	IpaB-F	GGACTTTTTAAAAGCGGCGG	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Invasion plasmid Antigen B (314)	31
	IpaB-R	GCCTCTCCCAGAGCCGTCTGG			
Pseudomonas spp.	PA-GS-F	GACGGGTGAGTAATGCCTA	2 min at 95°C; 25 cycles of 94°C for 20 s, 54°C for 20 s, 72°C for 40 s	16S rRNA (618)	32
	PA-GS-R	CACTGGTGTTCCTTCCTATA			
Campylobacter spp.	IC-F	CTAGAGTACAAACTAATAAGTCTC	3 min at 95°C; 30 cycles of 94°C for	Flanking regions of ITS	33

	IC-R	ATTCTAAAACGCATCACTTCCTTG	45 s, 52°C for 45 s, 72°C for 45 s	gene (700bp)	
Yersinia spp.	227Fmod	GTCTGGGCTTTGCTGGTC	5 min at 95°C; 40 cycles of 94°C for 20 s, 60°C for 20 s, 72°C for 15 s	*omp*F (428 - 465)	34
	669R	GCGTCGTATTTAGCACCAACG			
Klebsiella spp.	gryA-F	CGCGTACTATACGCCATGAACGTA	3 min at 95°C; 35 cycles of 94°C for 1 min, 50°C for 30 s, 72°C for 30 s	Gyrase A gene (383)	35
	gyrA-C	ACCGTTGATCACTTCGGTCAGG			
C. parvum	ExCry1	GCCAGTAGTCATATGCTTGTCTC	5 min at 95°C; 10 min 80°C, 45 cycles of 94°C for 45 s, 53°C for 1 min, 72°C for 45 s	18S rRNA ribosomal unit (840)	36
	ExCry 2	ACTGTTAAATAGAAATGCCCCC			
	NesCry3	GCGAAAAAACTCGACTTTATGGAAGGG	5 min at 95°C; 10 min 80°C, 35 cycles of 94°C for 25 s, 65°C for 1 min, 72°C for 25 s	18S rRNA ribosomal unit (590)	
	NesCry4	GGAGTATTCAAGGCATATGCCTGC			
G. lamblia	Gia2029	AAGTGTGGTGCAGACGGACTC	5 min at 94°C; 35 cycles of 94°C for 30s, 65°C for 30 s, 72°C for 1 min	16S-rRNA ribosomal unit (497)	37, 38
	Gia2150c	CTGCTGCCGTCCTTGGATGT			
	RH11	CATCCGGTCGATCCTGCC	5 min at 94°C; 40 cycles of 94°C for 30s, 55°C for 30 s, 72°C for 1 min	16S-rRNA ribosomal unit (292)	
	RH4	AGTCGAACCCTGATTCTCCGCCAGG			

Genomic DNA extractions from rainwater for the detection of *G. lamblia* and *C.parvum*.

For the detection of *G. lamblia* and *C. parvum*, rainwater samples were collected during the third sampling session from selected rainwater tanks in the Kleinmond housing scheme. The tank water samples were processed according to Dungeni et al. (43) with a few modifications. To extract total genomic DNA from the tank water samples, 5 L of each sample was filtered through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences) with a pore size of 0.8 µm and a diameter of 47 mm. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 70 kPa. The filters were then washed using 2 mL 0.1% Tween 80. After the filters had been removed, the samples underwent centrifugation (1000 × g) to pellet the (oo)cysts. Deoxyribonucleic acid extractions were then performed on the pellet using the QIAamp DNA® Stool Mini Kit (Qiagen, Hilden, Germany) (44) with minor modifications. Briefly, the pellet was re-suspended in 1.4 mL Buffer ASL and incubated at 95˚C for five minutes. Once the sample had undergone subsequent centrifugation (full speed for one minute), an InhibitEX tablet was added to 1.2 mL of the supernatant, vortexed for one minute and left at room temperature for one minute. In order to remove the inhibitors bound to InhibitEX the sample was centrifuged at full speed for three minutes. In a microcentrifuge tube containing 30 µL of Proteinase K, 400 µL of the supernatant was added. Before incubating the sample at 70˚C for 15 minutes, 400 µL of Buffer AL was added, and after incubation 400 µL of ethanol (96 to 100%) was added to each sample. In order to filter the complete lysate through the QIAamp spin column approximately 600 µL of the lysate was added to the QIAamp spin column and centrifuged at full speed for one minute. This was then repeated until the full lysate had been filtered through the QIAamp spin column. The protocol was then performed as per manufacturer's instructions.

Identification of *G. lamblia* and *C. parvum*.

The primers along with the PCR reactions utilized for the identification of Cryptosporidium parvum and Giardia lamblia have been previously described by Sturbaum et al. (36), Appelbee at al. (37) and Hopkins et al. (38). Primers used in this study are outlined in Table 4.1. The PCR mixture used to identify Cryptosporidium parvum consisted of a final volume of 50 µL. It included 10 µl of 5X Green GoTaq® Flexi Buffer (Promega) (1X), 2.5 µL of each PCR primer (0.5 µM) and 0.3 µL of GoTaq® Flexi DNA Polymerase (Promega) (1.5U). The PCR conditions for the external and nested PCR are outlined in Table 4.1, where a nested-PCR reaction was performed using 5 µL of the primary PCR reaction.

For the detection of the Giardia spp. in the tank water samples, a primary and semi nested PCR was performed (Table 4.1). Again, the PCR mixture consisted of a final volume of 50 µL. It included 10 µl

of 5X Green GoTaq® Flexi Buffer (Promega) (1X), 4 µL MgCl2 (2.0mM), 0.5 µL of each dNTP (0.1 mM) (Thermo Scientific), 2.5 µL of each PCR primer (0.5 µM) listed in Table 4.1, and 0.3 µL of GoTaq® Flexi DNA Polymerase (Promega) (1.5U). A secondary PCR mixture consisted of the same components, except that 0.5 µL of the primary PCR product was used in place of genomic DNA. A 292 bp product was amplified using the primers, RH11 and RH4 (Table 4.1). The secondary PCR assay was identical to the initial PCR, with the exception that the annealing temperature was increased to 59°C.

The secondary PCR products for both C. parvum and G. lamblia were subsequently cloned into pGEM T-easy vector system (Promega, Corp.) according to manufacturer's instructions and then transferred into E. coli DH5α and plated onto Luria-Bertani agar plates containing ampicillin and X-gal (5-bromo-4-chloro-3-indolyl-β-D-thiogalactopyranoside) as recommended by the manufacturer. Plasmid DNA was extracted from the clones using the PureYield™ Plasmid Miniprep System (Promega) and plasmids containing the correct size PCR product (2% agarose gel in 1X TBE) correlating to the band of *Giardia* (292 bp) and *Cryptosporidium* (590 bp) spp. were sent to Central Analytical Facility (CAF) at Stellenbosch University for sequencing.

Analysis of PCR products.

All PCR products were analyzed by gel electrophoresis in either 0.8% (16S rRNA identification), 1.5% (genus specific analysis) or 2% (PCR clones) agarose (Bio- Rad) containing 0.5 µg/mL ethidium bromide in TBE buffer. Once the size and the concentration of the PCR products had been confirmed, products of representatives of the bacteria detected in the tank water samples were cleaned and concentrated using the DNA Clean & Concentrator™-5 Kit (Zymo Research) as per manufacturer's instructions. The cleaned products were then sent to the CAF for sequencing. Sequences were aligned and analyzed using DNAmanTM version 4.1.2.1 software to form continuous sequences. Chromatograms of each sequence were examined using Finch TV v. 1.4.0 software and were aligned using DNAmanTM version 4.1.2.1 software. Sequence identification was completed using the National Centre for Biotechnology Information (NCBI) and The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest match of local similarity between isolates and the international database in GenBank, EMBL, DDBJ and PDB sequence data (45). The sequences of representative isolates, that showed > 97% similarity (< 3% diversity) to organisms were recorded.

Statistical analysis.

The genus specific PCR assays representing each of the pathogenic bacteria (*Shigella* spp., *Salmonella* spp., *Aeromonas* spp., *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Yersinia* spp.) were analyzed to determine relations between the detection specifics of each organism. Polymerase chain reaction results obtained from the *Campylobacter* PCR assays were not included, as no PCR assays showed positive results for *Campylobacter* spp. To analyze the data obtained from the genus specific PCR assays, a positive PCR product was assigned the value 1, and when no PCR product was observed, it was assigned the value 0. Binary logistic regression has previously been used to investigate water samples, as this model can be applied to presence/absence (binary) results (46). In order to validate the co-occurrence and non-co-occurrence results, the odds ratio, specificity and sensitivity of each PCR assay to detect the corresponding PCR assay were determined according to Gawler et al. (47).

RESULTS

Identification of dominant indigenous pathogenic bacteria based on 16S rRNA analysis.

From the 29 rainwater tanks that were sampled four times (one to four days after a rain event) throughout the study period during a higher rainfall period (74.7 mm in June to 198.1 mm in August), 85 isolates were selected from a larger number of colonies based on morphological characterization. All the organisms isolated throughout this study were predominantly Gram negative, except for *Enterococcus faecium* (Genbank accession no. AMBN01000001.1) isolated during sampling four which is a Gram positive organism. The organisms identified as well as the frequency of detection (percentage) throughout the sampling are summarized in Table 4.2.

Aeromonas spp. and Pseudomonas spp. were isolated throughout the high rainfall period (sampling occasions one to four). Enterobacter spp., Klebsiella spp. and Serratia spp. were also commonly identified species as these genera were identified in three (75%) of the four sampling occasions. During two of the four sampling occasions Providencia spp., Ochrobactrum spp., Raoultella spp., Citrobacter spp. and Acinetobacter spp. were isolated and identified. Chryseobacterium spp., Salmonella spp., Proteus spp., Comamonas spp., Morganella spp., Escherichia spp., Rahnella spp., Enterococcus spp. and Shigella spp. were only isolated and identified on one sampling occasion and represent the least prevalent genera.

TABLE 4.2. The various genera of bacteria isolated throughout the sampling period along with the frequency of occurrence percentage (n = 4)

Bacteria identified during the particular sampling times (percentage of occurrence)	
Aeromonas spp. (100%)	*Proteus* spp. (50%)
Pseudomonas spp. (100%)	*Chryseobacterium* spp. (25%)
Enterobacter spp. (75%)	*Salmonella* spp. (25%)
Klebsiella spp. (75%)	*Comamonas* spp. (25%)
Serratia spp. (75%)	*Morganella* spp. (25%)
Providencia spp. (50%)	*Escherichia* spp. (25%)
Ochrobactrum spp. (50%)	*Rahnella* spp. (25%)
Raoultella spp. (50%)	*Enterococcus* spp. (25%)
Citrobacter spp. (50%)	*Shigella* spp. (25%)
Acinetobacter spp. (50%)	

During sampling one, 51% of the total 85 isolates were obtained and during this sampling the most diverse array of Gram negative bacteria were also isolated (Fig. 4.1). Isolates presented in the first clade, belonging to the Enterobacteriales order and the *Enterobacteriaceae* family represented the largest order found in sampling session one. In contrast, the order of *Aeromonadales*, represented one family, the *Aeromonadaceae*. Two families were observed in the third clade of *Pseudomonadales*, namely the *Moraxellaceae* and *Pseudomonadaceae*. The order of *Rhizobiales* contained one genus of *Ochrobactrum* belonging to the *Brucellaceae* family. The families of *Comamonadaceae* and *Flavobacteriaceae* formed clades five and six, respectively.

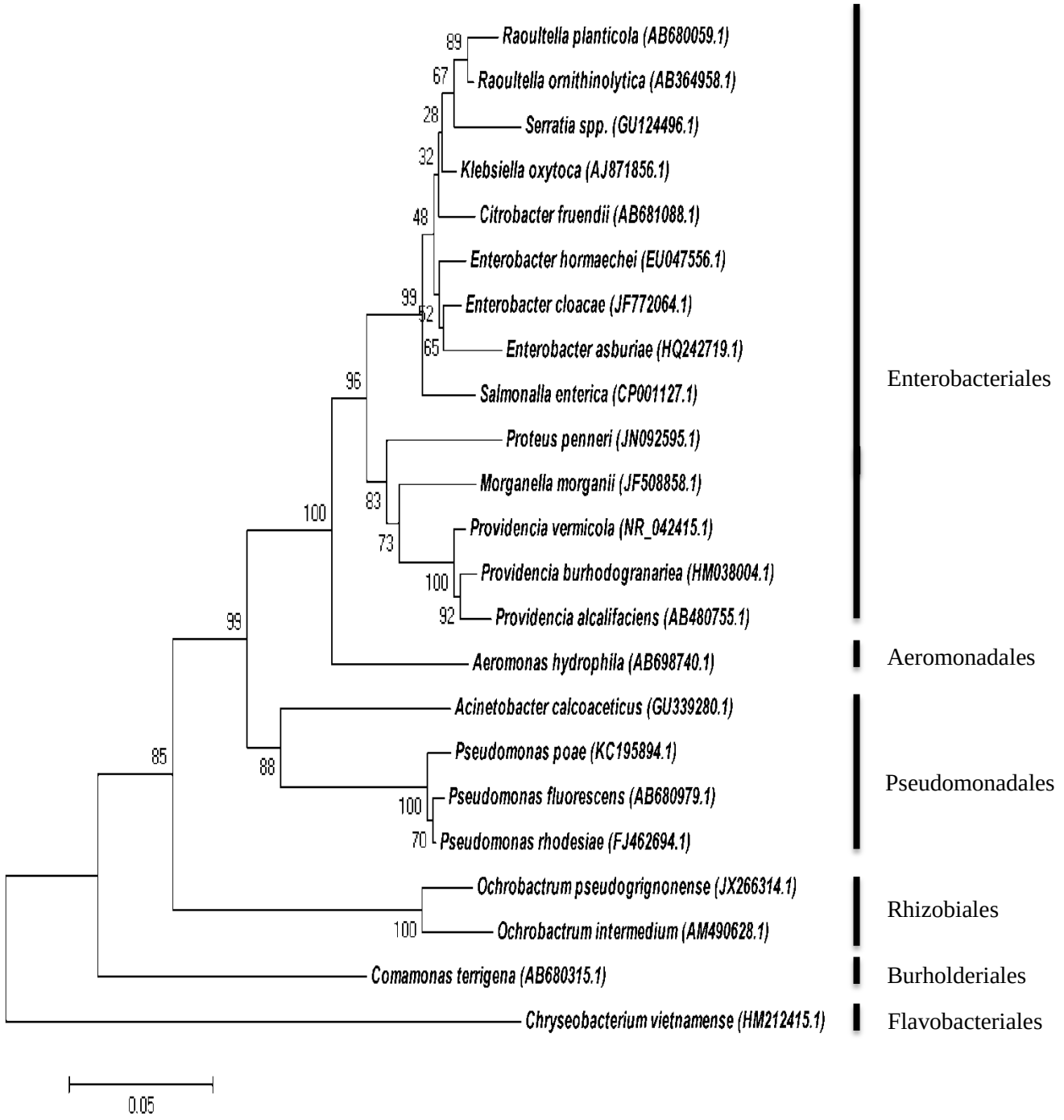

FIG 4.1. A phylogenetic tree illustrating the evolutionary history of bacteria was constructed during sampling session one, by means of the Maximum Composite Likelihood function (evolutionary history) and neighbour joining method in Mega 5. The numbers found adjacent to the nodes represent the percentages of 1000 exploratory bootstrap trials. The scale bar indicates 0.05 changes per site. Six distinct orders are clustered together representing the *Enterobacteriales*, *Aeromonadales*, *Pseudomonadales*, *Rhizobiales*, *Burholderiales* and *Flavobacteriales*. The increased amount in bacterial diversity may be attributed to the fact that isolates were obtain from two different selective media.

The cladogram depicted in Fig. 4.2 represents the range of organisms (27% of total isolates) identified during the second sampling session. Firstly, the *Enterobacteriales* encompassing the dominant family of *Enterobacteriaceae* are clustered together in the first clade. *Aeromonadales* form the second clade, and include a family of *Aeromonadaceae* with one *Aeromonas* spp. The third clade of the *Pseudomonadales* includes two families of bacteria, the *Moraxellaceae* and *Pseudomonadaceae* which includes the one *Acinetobacter* spp. through to the *Pseudomonas* spp., respectively. The *Enterobacteriaceae* represented the largest family during sampling two, although the widest range of species diversity could be seen amongst the *Pseudomonas* genus.

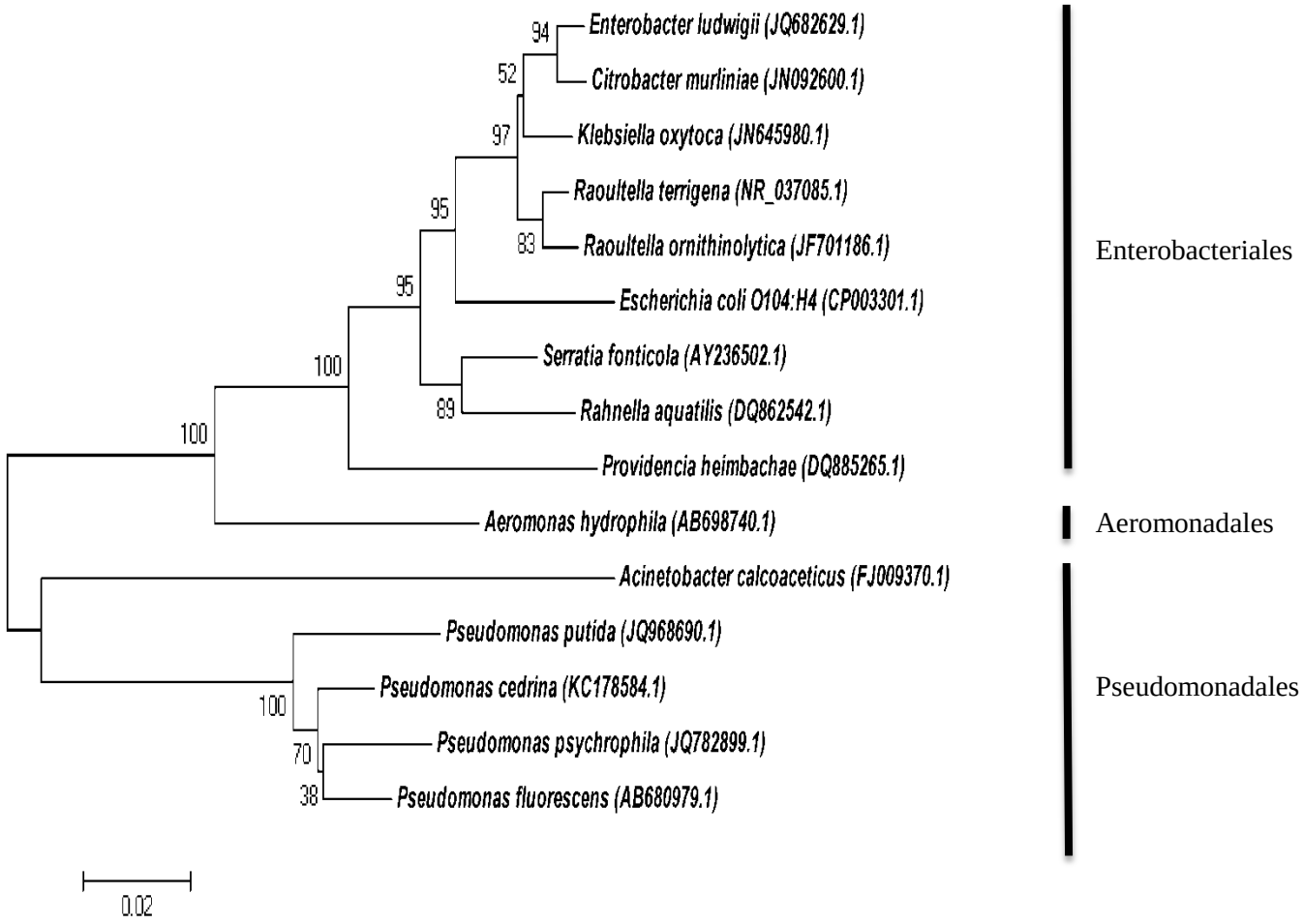

FIG 4.2. A phylogenetic tree illustrating the evolutionary history of bacteria was constructed during sampling session two, by means of the Maximum Composite Likelihood function (evolutionary history) and neighbour joining method in Mega 5. The numbers adjacent to the nodes represent the percentages of 1000 exploratory bootstrap trials. The scale bar indicates 0.05 changes per site and the tree is drawn to scale. Three distinct clades of *Enterobacteriales*, *Aeromonadales* and *Pseudomonadales* encompass the families of *Enterobacteriaceae*, *Aeromonadaceae*, *Moraxellaceae* and *Pseudomonadaceae*.

During the third and fourth sampling sessions low diversity amongst the bacteria was observed where 11 and 12% of the total isolates were recovered, respectively (data not shown). During the third sampling *Aeromonas* spp., *Pseudomonas* spp., *Enterobacter* spp. and *Klebsiella* spp. were isolated and identified, while the following species were isolated and identified during sampling four; *Aeromonas* spp., *Pseudomonas* spp., *Serratia* spp., *Enterococcus* spp. and *Shigella* spp.

Presence of potential pathogenic bacteria in tank water samples.

Genus specific PCR results are indicated in Table 4.3 for sampling sessions one to four. *Legionella* spp. (GenBank accession no. KC209478.1, FJ588216.1) were dominant in this study as, on average, this genus was detected in 73% of the tank water samples. The lowest number of positive samples, for *Legionella* spp., was detected during sampling three (41%), while the highest number of positive samples was identified in sampling four (86%). *Salmonella enterica* subsp. *enterica* serovar Gallinarum/pullorum were the least dominant species as on average only 6% of the samples tested positive for *Salmonella* spp. (GenBank accession no. CP003786.1). The highest number of positive results for *Salmonella* spp. was observed during sampling two (17%) while no *Salmonella* spp. were detected during samplings one and three. An average of 47% of all samples tested were positive for *Klebsiella pneumoniae* and *Klebsiella oxytoca* (GenBank accession no. X16817.1, FM199942.1), with the highest value recorded in sampling two (75%) and the lowest value recorded in sampling three (24%). *Shigella* and *Yersinia* genus specific PCRs showed average positive results of 27% and 28%, respectively. The highest number of positive results was recorded in sampling four (45%) for *Shigella sonnei* (GenBank accession numbers. HE616529.1, HE616528.1) and sampling two (48%) for *Yersinia enterocolitica* (GenBank accession no. HM142628.1). In addition, the lowest number of *Shigella* spp. and *Yersinia* spp. positive results were detected in sampling three (10%) and sampling one (14%), respectively. An average of 16% and 13% was recorded for *Aeromonas hydrophila* (GenBank accession no. EF189591.1) and *Pseudomonas syringae* (GenBank accession no. KC920926.1, KF500097.1), respectively. No *Campylobacter* spp. were detected throughout the sampling period.

TABLE 4.3**.** The number and percentage of samples that contained specific genes linked to the genera analyzed for sampling one to four

Presence of genus specific genes in samples tested (n= 29) (%)								
Sampling Session	*Aeromonas* spp.	*Campylobacter* spp.	*Klebsiella* spp.	*Legionella* spp.	*Pseudomonas* spp.	*Salmonella* spp.	*Shigella* spp.	*Yersinia* spp.
1	4 (14)	0 (0)	11 (38)	24 (83)	0 (0)	0 (0)	11 (38)	4 (14)
2	7 (24)	0 (0)	22 (75)	24 (83)	12 (41)	5 (17)	4 (14)	14 (48)
3	2 (7)	0 (0)	7 (24)	12 (41)	2 (7)	0 (0)	3 (10)	6 (21)
4	5 (17)	0 (0)	14 (48)	25 (86)	1 (3)	2 (7)	13 (45)	9 (31)

Presence of *G. lamblia* and *C. parvum* in harvested rainwater samples.

During the third sampling session randomly selected tanks were sampled in order to detect the protozoan species *Giardia* and *Cryptosporidium*. The nested-PCR assay confirmed that during the third sampling session, two out of the eight tanks sampled contained *Giardia intestinalis* (GenBank accession no. KC556751.1). No *Cryptosporidium* spp. were detected during the current study.

Levels of agreement, disagreement, odds ratio, specificity and sensitivity between genus-specific PCR assays.

The occurrences of frequently identified pathogens with genus specific PCR assays were analyzed with the use of a pairwise comparison for all the tank water samples. The pairwise comparisons that were significant ($p < 0.05$) are indicated in Table 4.4. The total agreement percentage was calculated by adding the percentage of co-occurrence and non-co-occurrence for each pairwise comparison. *Legionella* spp. and the *Shigella* spp. had the highest percentage (24%) co-occurrence agreement, while *Aeromonas* spp. and *Salmonella* spp. had the lowest percentage (3%) co-occurrence agreement. *Salmonella* spp. also had a co-occurrence agreement with *Klebsiella* spp. (5%), while *Pseudomonas* spp. had co-occurrence agreements with *Klebsiella* spp. (10%) and *Yersinia* spp. (7%).

The non-co-occurrence percentages were generally higher than the co-occurrence agreements, for most pairwise comparisons. However, *Legionella* spp. and *Shigella* spp. showed equal (24%) co-occurrence and non-co-occurrence agreements. *Aeromonas* spp. and *Salmonella* spp. had the highest (82%) non-co-occurrence agreement, while *Legionella* spp. and *Shigella* spp. had the lowest non-co-occurrence agreement. *Klebsiella* spp. had non-co-occurrence agreements with *Pseudomonas* spp. (50%) and *Salmonella* spp. (52%), while the non-co-occurrence agreement between *Pseudomonas* spp. and *Yersinia* spp. was 66%.

To validate the presence of one PCR assay indicating the positive or negative result of another PCR assay, the sensitivity and specificity of each PCR assay was determined for all the PCR assays showing the most significant co-occurrence and non-co-occurrence between samples (Table 4.4). To determine the level of association or non-independence of the PCR assays for each bacteria pair, the odds ratio was included in this study. The highest overall sensitivity value was recorded for the *Legionella* spp. vs. *Shigella* spp. PCR assays at 0.90 (odds ratio: 0.95) followed by *Klebsiella* spp. vs *Salmonella* spp. (0.86; odds ratio: 0.99). The highest specificity for the PCR assays was also recorded for *Pseudomonas* spp. vs. *Yersinia* spp. at 0.92 (odds ratio: 0.62) followed by *Aeromonas* spp. vs. *Salmonella* spp. (0.87; odds ratio: 0.99).

TABLE 4.4. Total agreement and total disagreement on the co-occurrence and non-co-occurrence amongst bacterial genes detected in rainwater samples using genus specific PCR assays (n=116) and the sensitivity, specificity and odds ratio of PCR assays

Pairwise comparison	Co-occurrence agreement (%)	Non-co-occurrence agreement (%)	Total agreement (%)	Total disagreement (%)	P value	Sensitivity	Specificity	Odds ratio
Klebsiella spp. vs. *Pseudomonas* spp.	10	50	60	40	0.024	0.73	0.57	0.98
Klebsiella spp. vs. *Salmonella* spp.	5	52	57	43	0.026	0.86	0.56	0.99
Aeromonas spp. vs. *Salmonella* spp.	3	82	85	15	0.008	0.57	0.87	0.99
Legionella spp. vs. *Shigella* spp.	24	24	48	52	0.007	0.90	0.33	0.95
Pseudomonas spp. vs. *Yersinia* spp.	7	66	73	27	0.029	0.24	0.92	0.62

DISCUSSION

Based on 16S rRNA analysis, the majority of *Pseudomonas* isolates were obtained during sampling two, while the lowest number were obtained during sampling four. This correlated well with the genus specific PCR where it was revealed that most of the tank water samples tested positive for the presence of *Pseudomonas* spp. during sampling two (40%). However, no *Pseudomonas* spp. were detected using genus specific primers in sampling one with 3% identified during sampling four. *Pseudomonas* spp. have previously been identified in rainwater samples, for example Uba and Aghogho, (11) found that 83% of the rainwater samples tested were positive for *Pseudomonas* spp.

Pseudomonas spp. represent the most diverse array of species and were isolated (16S rRNA PCR) throughout the sampling period, including presumptive *P. poae* (sampling one), *P. fluorescens* (sampling one, two and three), *P. fragi* (sampling one), *P. putida* (sampling two and four), *P. reactans* (sampling eight), *P. rhodesiae* (sampling one and four), *P. cedrina* (sampling two) and *P. psychrophila* (sampling two). This is not surprising as *Pseudomonas* spp. have been shown to be abundant in water and soil ecosystems (48). This genus has however, been associated with significant plant, human and animal diseases and has been well documented in the spoilage of food products such meat, even under refrigeration conditions (49, 50).

Aeromonas hydrophila was detected in every sampling session, utilizing 16S rRNA analysis. Various other *Aeromonas* spp., for example, *A. caviae* (sampling three) and *A. salmonicida* (sampling four) were also identified during the sampling period. Based on genus specific PCR analysis *Aeromonas* spp. were also present in all of the sampling sessions. The majority of the *Aeromonas* isolates (16S rRNA) were obtained during sampling four, while genus specific PCR revealed that the majority of the rainwater samples contaminated with *Aeromonas* species were detected during sampling two (24%). Both 16S rRNA and genus specific PCR revealed that the lowest concentration of *Aeromonas* spp. was identified during sampling three (7%). In the current study, presumptive *A. hydrophila* and *A. caviae* were of clinical significance (51). *Aeromonas* spp. cause a wide range of diseases, including gastrointestinal infections as well as extraintestinal infections, such as cellulitis, wound infections, septicemia, urinary tract infections, hepato-biliary infections, and ear infections (52). The *Aeromonas* spp. most commonly associated with gastrointestinal infections is *A. hydrophila* (53). *Aeromonas* spp. have also been identified in previous studies that monitored the microbial quality of harvested rainwater (9, 10, 54).

Along with *Aeromonas*, *Klebsiella* spp. [including presumptive *K. oxytoca* (sampling one and two), *K. cryocrescens* (sampling three) and *K. intermedia* (sampling four)] were also isolated

and identified in the current study using 16S rRNA PCR. *Klebsiella* spp. were predominantly identified in sampling session one using 16S rRNA PCR, while genus specific PCR showed that this genus was present in higher concentrations during sampling two (75%). No *Klebsiella* isolates (16S rRNA PCR) were obtained during sampling four while the least amount of positive samples (genus specific PCR) were observed during sampling three (24%). *Klebsiella oxytoca* has been isolated on numerous occasions from Australian drinking water sources (55). Kaushik et al. (56) also showed recently that of the 50 rainwater samples tested in Singapore, 12% were positive for *Klebsiella* spp. People that are at added risk of acquiring *Klebsiella* associated diseases are, amongst others, those with compromised defense mechanisms, people with serious wounds, those with Acquired Immune Deficiency Syndrome (AIDS), or immune-compromised HIV-infected adults (57).

Salmonella isolates were only positively identified in sampling one (*S. enterica*) using 16S rRNA, while most of the samples yielded positive results for *Salmonella* during sampling two utilizing genus specific PCR. No *Salmonella* isolates (16S rRNA) were obtained from sampling two to four, while no samples showed positive results for *Salmonella* during sampling one and three (genus specific PCR). Salmonellosis waterborne outbreaks are usually as a result of the consumption of water that has been contaminated by the feces of livestock and native animals or by wastewater discharges that have been inadequately treated. Koplan et al. (58) found that there was an outbreak of salmonellosis amongst a church group after the ingestion of harvested rainwater contaminated with *S. arechevalata*. It was also hypothesized that this was as a result of the catchment area (roof) being covered with bird feces.

No *Yersinia* spp. were identified throughout the study period using 16S rRNA analysis, however *Yersinia* spp. were identified in all the sampling sessions with the use of genus specific PCR. This could be attributed to the use of PCR based assays, whereby the detection of bacteria in a viable but non-culturable state is possible (59). Moreover, genus specific PCR indicated that the highest amount of samples (48%) tested positive for *Yersinia enterocolitica* during sampling two, while sampling one yielded the lowest percentage of positive samples (14%). *Yersinia enterocolitica* have been predominantly isolated from many domestic and wild animals, which are considered possible reservoirs. Transmission of this bacterium is generally through meat products (60), however *Y. enterocolitica* has been isolated from a number of environmental samples, particularly from water, but the serotypes are different to those that cause disease in humans.

Legionella spp. were predominantly detected throughout the sampling sessions with the use of genus specific PCR assays. However, no *Legionella* spp. were isolated and identified with the

use of culturing techniques and 16S rRNA sequencing. While an alternative to the serial dilution method (employed in the current study), would be to filter larger volumes of tank water through a membrane filter, research has shown that *Legionella* spp. are difficult to isolate using culture based techniques (3), especially from low nutrient environments (61). High incidence of *Legionella* was detected during sampling four (86.2%), one (82.8%) and two (82.8%), while the lowest percentage of positive results was obtained during sampling three. The use of quantitative PCR has also enabled researchers in other studies to observe and quantify the estimated numbers of *Legionella pneumophila* in harvested rainwater samples (9, 16).

Of the isolates obtained, *Enterobacter* spp., which included *E. hormaechei* (sampling one), *E. cloacae* (sampling three) and *E. ludwigii* (sampling two and four), were isolated throughout the sampling period. *Enterobacter* spp. represents the fourth most dominant genera of isolates obtained in this study, after *Pseudomonas*, *Aeromonas* and *Klebsiella*.

During this study, of the eight tank water samples analyzed, *Giardia* spp. were detected in 25% of the rainwater tanks sampled, however, no *Cryptosporidium* spp. were detected in rainwater sampled from the same tanks. *Giardia* and *Cryptosporidium* are protozoan parasites. In a previous study, Ahmed et al. (16) did not detect the presence of *Cryptosporidium* spp. with the use of PCR based assays, however in a later publication (24) with the use of quantitative PCR (qPCR) they were able to establish that *Cryptosporidium parvum* and *Giardia lamblia* were present in 13% and 30% of the possum fecal samples tested, respectively, and *G. lamblia* was present in 13% of the rainwater samples analyzed.

Statistical analysis revealed that *Klebsiella* spp. vs. *Pseudomonas* spp. had a total agreement of 60%, while *Klebsiella* vs. *Salmonella* had a total agreement of 57%. *Klebsiella* and *Pseudomonas* are not associated with the gastrointestinal system, and *Klebsiella* is known to propagate in the environment. Both these genera (*Klebsiella* spp. and *Pseudomonas* spp.) do not serve as indicators of fecal contamination, while *Salmonella* does (62). As there is not much variation amongst the total agreement values, the presence of *Klebsiella* and possibly *Pseudomonas*, could serve as indicators for the presence of *Salmonella* and fecal contamination. However, further analysis revealed that the results could be misleading as the specificity of the test was only 0.57 (*Klebsiella* spp. vs. *Pseudomonas* spp.) and 0.56 (*Klebsiella* spp. vs. *Salmonella* spp.). A similar scenario is mimicked for *Legionella* spp. vs. *Shigella* spp. where a total agreement of 48% was obtained. As the total agreement (48%) and total disagreement (52%) values were similar, it is hypothesized that *Legionella* spp. could not serve as an indicator for the presence of *Shigella* spp. contamination, as the specificity of the assay was 0.33. However, *Legionella* is not associated with the gastrointestinal

system, while *Shigella* spp. serve as an indication of fecal pollution in a water sample and may pose a significant health risk (62).

However, *Aeromonas* spp. vs. *Salmonella* spp. had a high total agreement and a lower total disagreement of 85% and 15% respectively, with *Pseudomonas* vs. *Yersinia* also having a high total agreement and a lower total disagreement of 73% and 27%, respectively. *Aeromonas* and *Pseudomonas* are normal inhabitants of fresh water, whereas, the presence of *Salmonella* in a water sample indicates fecal contamination, and the presence of *Yersinia* indicates the presence of domestic or wild animal fecal contamination (62). Using these two PCR assays, *Aeromonas* spp. vs. *Salmonella* spp. and *Pseudomonas* spp. vs. *Yersinia* spp., to monitor fecal pollution would pose less chance of misleading results as the specificity of both tests was high, 0.87 and 0.92, respectively, and above the U.S Environmental Protection Agency (EPA) recommended value of 0.80 (63).

Although the PCR results do not indicate the pathogenicity of the particular bacteria, the results of the PCR assays in this study show that a particular PCR assay can be used to detect the presence or absence of another organism in a PCR assay, for example the PCR assay for *Salmonella* spp. could determine the presence or absence of *Aeromonas* spp. as the specificity value (0.87) and the odds ratio for this pair of organisms was high (0.99). Further research is however, essential to determine the prevalence and concentrations of each genus specific gene preferably in a quantitative manner. As previously noted by Ahmed et al. (46), the consequences of inadequate and misleading analysis for the detection of bacterial pathogens could lead to expensive treatment methods that may not be assessed efficiently rendering the quality of the water inadequate or, as in the case of observing false positive results, the water could wrongfully be deemed inadequate and allow for overly restricted access to the water source.

A range of dominant and indigenous predominantly Gram negative potential pathogenic bacteria, for example, *E. aerogenes* and *S. enterica*, were isolated and identified, with the use of 16S rRNA analysis in the harvested rainwater sampled throughout the study period. Moreover, PCR assays utilizing genus specific primers confirmed the presence of bacterial and protozoan groups that have been detected internationally in harvested rainwater samples, such as *Aeromonas* spp., *Salmonella* spp., *Legionella* spp., *Yersinia* spp. and *Giardia* spp. It was also important to note that the diversity and frequency of distribution of the pathogenic species detected differed significantly amongst the 29 rainwater tanks sampled with no correlation observed over the four sampling occasions. Results obtained in the current study then correlate with previous studies where it was noted the method of catchment of harvesting rainwater from rooftops, courtyards or low frequented streets, could allow pathogens from animal droppings and other organic debris to be flushed into the

tanks via the gutters and inlet tank system. At the Kleinmond sampling site, the houses are also surrounded by gardens and a dirt road that runs along the top of the settlement which could allow pathogens from the environment (soil and water) or fecal matter to enter the tank reservoir. This is a matter of concern as in a study conducted by P. H. Dobrowksy, D. Mannel, M. De Kwaadsteniet, H. Prozesky, W. Khan and T. E. Cloete (unpublished data) in the same Kleinmond settlement, total coliforms and Escherichia coli counts enumerated in rainwater samples significantly exceeded ($p < 0.05$) the stipulated drinking water guidelines during every sampling session. Moreover, a social perception study was also conducted in the same study, and it was noted that, generally, the harvested rainwater was used for washing clothes and for cleaning inside and outside the houses although 24% of the respondents used the water for drinking. The results of these studies as well as the confirmed presence of pathogenic bacteria and protozoa, then reinforces the opinion of the WHO (64), which recognises that rainwater should be pre-treated before using it as a potable source, especially if the rainwater is to be used by immunocompromised individuals.

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Chapter 5:

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(Chapter 5 is thus compiled in the format of the Water Research Commission report and UK spelling is employed)

Efficiency of PVA Nanofibre, Activated Carbon and Slow Sand Filtration Systems in Removing Chemical and Microbial Contaminants from Harvested Rainwater

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Abstract

Numerous studies have indicated that harvested rainwater should not be used for potable purposes without prior treatment. The aim of this study was thus to evaluate the efficiency of four household point-of-use treatment systems, namely, activated carbon, PVA nanofibre column, slow sand filtration and a activated carbon/PVA nanofibre column, for the treatment of harvested rainwater. Three rainwater harvesting tanks were installed on the Welgevallen Experimental farm, Stellenbosch, South Africa, with the respective filtration systems attached to two tanks intermittently throughout the study period. Parameters used to monitor the four filtration systems included, amongst others, metal ion, cation and anion analysis as well as heterotrophic bacteria, *Escherichia coli* and total coliform enumeration. Chemical analyses indicated that while numerous cation and anion concentrations were within drinking water guidelines in the unfiltered and filtered rainwater, the concentrations of isolated cations, such as aluminium, antimony, manganese and iron, increased after filtration through the respective filtration systems. Slow sand filtration, slow activated carbon filtration and a PVA nanofibre (without activated carbon) column were also not effective in removing the microbiological load of harvested rainwater according to drinking water guidelines. Moreover, it was observed that one litre of potable water per 37.41 minutes could be provided by the activated carbon/PVA nanofibre column. However, PCR assays indicated that *Klebsiella* spp. *Legionella* spp., *Pseudomonas* spp. and *Yersinia* spp. were not removed by the activated carbon/PVA nanofibre column. Further studies will be conducted to quantify the organisms which persisted after filtration though the activated carbon/PVA nanofibre column and to determine whether these pathogens were viable.

Key words: Microfiltration; biofiltration; indicator bacteria; chemical quality; rainwater harvesting

5.1 Introduction

In an updated report compiled by the WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation, the achievements of the Millennium Development Goals towards achieving Target 7c were recorded. The initial target was to half the proportion of people without sustainable access to safe drinking water and basic sanitation. It was reported that the target had been met in 2010, however, over 780 million people worldwide still remain without access to potable water, predominantly in the developing countries (WHO/UNICEF, 2012). With the aim to eliminate the load of disease caused by substandard drinking water, rainwater harvesting has been proposed as an alternative technology to provide an adequate, safe and inexpensive water supply to households (Theron and Cloete, 2002; Ashbolt, 2004; Eshelby, 2007). However, many studies have indicated that the microbiological quality of harvested rainwater does not comply with drinking water standards and if not treated, could pose a serious health risk (Spinks et al., 2006; Ahmed et al., 2008; Lee et al., 2010; Ahmed et al., 2011).

Smaller communities are at a greater risk in receiving water supplies of poor quality as in most instances the water managers lack experience and are unable to maintain and upgrade the water supply facilities (Mwabi et al., 2011). Therefore a cost effective and simple point-of-use household treatment method for harvested rainwater may provide a manner in which households and communities can gain access to drinking water. The use of slow sand filters is not a new concept as for over 150 years slow sand filters have been effective in treating water and preventing the spread of gastrointestinal disease (Logsdon et al., 2002). Recent improvements to the slow sand filtration process have allowed this technology to be used more widely, and as previously noted the filtration systems are especially beneficial in treating water from small water systems (Logsdon et al., 2002). The effective removal of microorganisms from water relies on the development of a well-established biofilm layer known as the schmutzdecke and biological processes aid in the successful functioning of the slow sand filtration process (Schulz and Okun, 1983). In addition, studies have shown that slow sand filtration is effective in removing viruses (Yahya et al., 1993), bacteria and protozoa (Hijnen et al., 2007) from water sources.

Granular activated carbon filtration has proved to be useful where conventional methods in treating water such as coagulation, sedimentation and filtration have been insufficient in lowering for example, chemical oxygen demand and ammonium concentrations (Zhang et al., 2013). To remove contaminating substances and improve the water quality the system relies on the immobilization of bacteria onto the surface of granular activated carbon which in turn is able to remove pollutants *via* processes such as bio-absorption, biodegradation or bio-regeneration (Wang et al., 2007; Ong et al.,

2008). Granular activated carbon covered with bacteria is known as biological activated carbon (BAC) and many studies have shown that BAC is efficient in removing dissolved organic matter and ammonium (Andersson et al., 2001; Tian et al., 2009).

Membrane filtration is a process that is able to remove particles and bacteria by filtering fluid through a micro-porous membrane with average pore sizes ranging from 0.1 to 10 μm (Li et al., 2010). Due to the ease of operation and high efficiency of filter membranes, numerous pressure-driven membrane filtration processes have arisen, for example microfiltration, ultrafiltration, nanofiltration and reverse osmosis (Geise et al., 2010; Kavitskaya et al., 2010). The efficiency of the filter membrane in removing impurities during water purification may depend on, for example, the presence of small colloidal particles that may interfere in the removal of bacteria due to size exclusion (Wang et al., 2013). Depending on the scaffold used to construct the membrane filter, studies have indicated that bacteria and viruses may be effectively removed from a water source using these systems (Wang et al., 2013).

The aim of this study was to assess the efficiency of biological filtration and microfiltration in the treatment of rainwater collected directly from domestically harvested rainwater (DRWH) tanks. Four household water treatment systems were evaluated: two biological filtration systems namely, the slow sand filter and the slow activated carbon filter and two microfiltration systems namely, a polyvinyl (alcohol) (PVA) nanofibre membrane column and a PVA nanofibre membrane/activated carbon column. The microbial parameters that were investigated included faecal and total coliforms, *Escherichia coli* and heterotrophic bacteria. In addition, the treated and untreated rainwater samples were screened for the presence of selected pathogenic bacteria using molecular techniques. The chemical parameters that were investigated included the concentration of metal ions, anions and cations present in the treated and untreated harvested rainwater samples.

5.2 Materials and Methods

5.2.1 Sample Site and Collection

Three polyethylene domestic rainwater harvesting (DRWH) tanks (2000 L) were installed at the Welgevallen Experimental farm, Stellenbosch University, South Africa (Figure 5.1). The solar pasteurization system was connected to tank A (discussed in Chapter 6), while the middle tank (tank B) and the right end tank (tank C) were used to test the filtering systems utilised in the present study. The farm is situated on the periphery of the town of Stellenbosch. As indicated in Figure 5.1, the sampling site was surrounded by trees; however no tree branches obstructed the catchment area. The farm was also surrounded by dirt roads that were continuously used by motor vehicles and the farm workers to herd cattle twice a day as the tanks were situated on the northern side of a well-

established building that neighboured the farms' dairy. In addition, due to damage, a section of the guttering lengths on the building where the DRWH tanks were installed had to be replaced, and new down pipes were installed in order to link the tanks to the old gutter system surrounding the catchment area. The older gutter system was constructed from white asbestos (Chrysotile) and the roofing was constructed from corrugated galvanized iron.

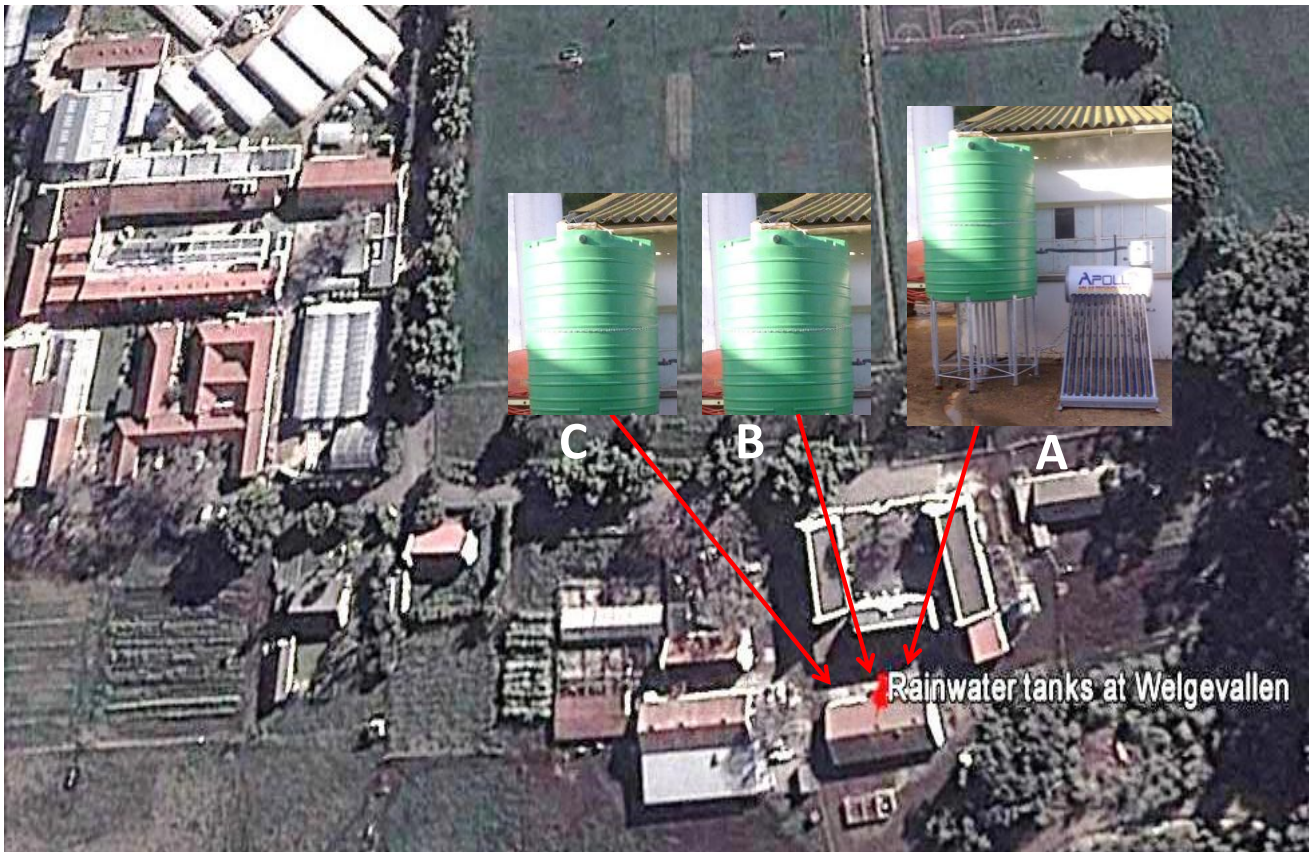


Figure 5.1. The sampling site (33°56'36.19"S, 18°52'6.08"E) used in the study was located at Welgevallen Experimental farm, Stellenbosch, South Africa. Three domestic rainwater harvesting tanks were installed: (A) Solar pasteurization system and (B) and (C) where various treatment systems were intermittently connected.

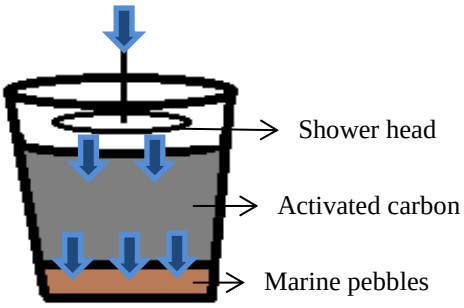
For microbial and chemical analysis, before and after treatment rainwater samples were collected in 1 L sterile Schott bottles. The temperature and pH of the rainwater at the sampling locations were measured using a hand-held mercury thermometer and colour-fixed indicator sticks with a pH range of 0 - 14 (ALBET®, Barcelona, Spain). Rainfall and temperature patterns were obtained from the South African Weather Services (SAWS, 2013), with samples collected from June to September 2013.

5.2.2 Point of use filtering systems employed for the treatment of rainwater

5.2.2.1 Biological Filtration: Activated Carbon

Two activated carbon filtration systems were set up directly next to rainwater tanks B and tank C (Figure 5.1). The filtering systems consisted of a smaller container (20 L) containing holes at the bottom, which fitted into a larger container (25 L) with a tap connected for effluent collection (Figure 5.2). The smaller container was filled with a bottom layer of 5 cm marine pebbles and then a top layer of approximately 17 cm of activated carbon (Aquasorb Udectrading Pty. Ltd) which represented the filtering material. A garden hose was then connected to the tap of a DRWH tank on the one end, and then to a shower head (20 cm diameter) on the other end. A shower head was fitted to the lid of the smaller container to allow the harvested rainwater to trickle through the shower head and over the activated carbon filtering medium. A biofilm was allowed to establish in the slow activated carbon filtration system by filling the system with rainwater and then allowing the system to remain closed for one week. The system was then drained and in a continuous flow arrangement, the rainwater was allowed to flow through the system for the duration of sampling and subsequent filtrate analyses.

A.



B.

Figure 5.2. (A) Schematic diagram of the smaller container containing marine pebbles and activated carbon which fitted into a larger container allowing for a slow activated carbon filtration system. (B) Photograph of the slow activated carbon system.

To determine the degree of chemical and bacterial pollution, samples were collected for five consecutive days. On each sampling day a 1 L water sample was collected directly from rainwater tanks B and C (before sample), respectively, and a 1 L rainwater sample was collected from the filtrate of the activated carbon filtration systems (an after sample) connected to tanks B and C, respectively.

5.2.2.2 Biological Filtration: Slow Sand Filtration

Two slow sand filtration systems were set up directly next to rainwater tanks B and tank C (Figure 5.1). The same system utilised for the activated carbon filtration (section 5.2.1 and Figure 5.2) was constructed with the exception that the slow sand filtration system consisted of a 5 cm layer of marine pebbles, placed in the bottom of the smaller container and approximately 22 cm of 0.61 mm silica sand (Cape Silica Suppliers CC, Cape Town, South Africa) placed on top of the pebbles. A biofilm was also allowed to establish for two weeks in a closed system before sampling took place by allowing water from the respective DRWH tanks to flow through the system. After the biofilm had formed, samples were collected every second day, for a total of six sampling events over a two week period. The water was allowed to continuously flow through the system between samplings. On each sampling day a 1 L water sample was collected directly from rainwater tanks B and C (before sample), respectively and a 1 L rainwater sample was collected from the filtrate of the slow sand filtration systems (an after sample) connected to tanks B and C, respectively.

5.2.2.3 Polyvinyl (alcohol) (PVA) nanofibre membrane filtration system

Polyvinyl (alcohol) nanofibres were produced by a process of needleless electrospinning utilising a Nanospider 200 Lab (Elmarco, s.r.o., Czech Republic). The substrate material onto which the nanofibres were deposited was a Tyvek material (Marshall Hinds, Johannesburg, South Africa) which was wound onto a core. A PVA polymer solution was made up by dissolving a PVA powder (Nippongohsei, Japan) in distilled water at 80˚C. The PVA polymer solution was modified by adding a cross-linker, acid and $CuCl_2$ (proprietary information). The PVA polymer solution was then poured into a polypropylene tub containing a stainless steel spinning electrode which was then partially submerged in the polymer solution. In order to create an electric field, a high voltage was connected to the spinning electrode with the collecting wire electrode grounded to create a potential difference. The spinning conditions were as follows; spinning distance was 100 mm, rotation speed of electrode was 3.2 rpm, high voltage was 80 kV, relative humidity was below 40% and speed of fabric was 0.1 m/min. Once the nanofibres were spun onto the Tyvek material, the newly synthesised membrane was cross-linked at 140°C for 15 min. A section of the membrane was analysed using scanning electron microscopy (SEM) at the Central Analytical Facility (CAF),

Stellenbosch University. Microscopy was performed using a LEO 1450VP SEM (Zeiss, Germany). The final product, a PVA nanofibre membrane was then used in a column flow through system.

The column system that was directly attached to tanks B and C is indicated in Figure 5.3. A schematic diagram of the PVA nanofibre membrane column is represented in Figure 5.3A, where unfiltered rainwater (red arrows) was allowed to flow through the PVA nanofibre membrane to the centre of the column and then filtered rainwater (blue arrows) was collected. The column systems were designed as follows, an inner cylinder containing holes (Figure 5.3B) was fitted inside a larger column (Figure 5.3C, D). A PVA nanofibre membrane was then wrapped around the inner cylinder twice which was then covered with a red netting (Figure 5.3E). This PVA nanofibre membrane system was assessed for bacterial removal efficiency only (Figure 5.3F).

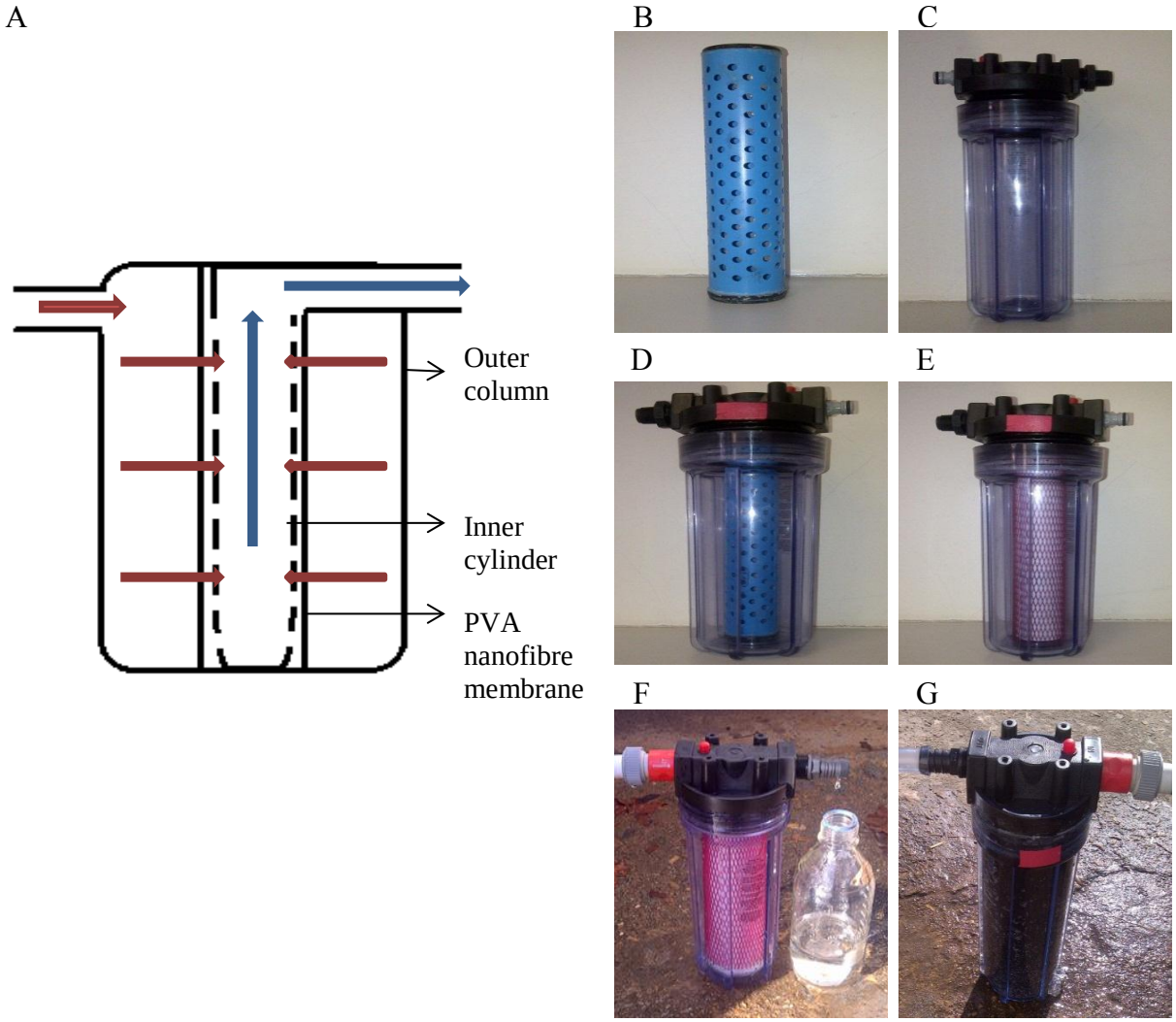


Figure 5.3. A) A schematic diagram of the PVA nanofibre membrane column, where unfiltered rainwater (red arrows) was allowed to flow through the PVA nanofibre membrane to the centre of the column and then filtered rainwater (blue arrows) was collected. B – F) A column system containing a PVA nanofibre membrane. G) Activated carbon was then layered around the PVA nanofibre membrane.

To determine the bacterial and chemical contamination removal efficiency, activated carbon (Aquasorb udectrading Pty. Ltd) was then layered around the PVA nanofibre membrane in order to exclude larger contaminants before passing through the PVA nanofibre membrane (Figure 5.3G). An initial 1 L rainwater sample was collected directly from tanks B and C. Five 1 L samples were then individually collected after the water passed through the PVA nanofibre membrane/activated carbon filtration system. The PVA nanofibre membrane and activated carbon was then replaced and another sampling was repeated with the system connected to each tank.

5.2.3 Chemical Analysis

Filtered samples collected from the slow sand and activated carbon biological filtration systems (before and after biofilm formation) and the PVA activated carbon column filtration system were analysed for the following chemical parameters. For the determination of the metal concentrations, Falcon™ 50 mL high-clarity polypropylene tubes containing polyethylene caps, were pre-treated with 1% nitric acid before sampling. The concentrations of metals such as aluminium (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn), amongst others, were then determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) according to Saleh et al. (2000) and nitric acid digestion. All samples were analysed for the presence of metals at the Central Analytical Facility (CAF), Stellenbosch University. The filtered rainwater samples were also sent to the Centre for Scientific and Industrial Research (CSIR) Stellenbosch for anion analyses. The anions detected along with the corresponding detection method are summarised in Table 5.1.

Table 5.1. Methods used in the detection of anions performed by the CSIR, Stellenbosch

Anion	**Method**
Nitrate and Nitrite	SALM 7.0 Automated Colorimetry
Soluble phosphate	SALM 9.0 Automated Colorimetry
Sulphate	MALS 6.5 ICP OES Detection
Chloride	SALM 1.0 Automated Colorimetry
Fluoride	SALM 11 Potentiometric measurement

5.2.4 Recovery of indicator organisms

To enumerate the heterotrophic bacterial count (HPC), a serial dilution was prepared for each sample (10^{-1}–10^{-2}) and by use of the spread plate method 100 µl of each dilution was cultured onto R2A agar (Difco) in triplicate, with the plates incubated at 37°C for up to four days. Total coliforms (TC) and *Escherichia coli* (*E. coli)* were then enumerated simultaneously by filtering a total volume of 100 mL (undiluted and 10^{-1}) through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The

filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (10 kPa, 10 psi). The filters were then incubated on Membrane Lactose Glucuronide Agar (MLGA) (Oxoid, Hampshire, England) at 35 ± 2°C for 18 - 24 hours (U.S. Environmental Protection Agency, 2009). All analyses were performed in triplicate.

5.2.5 The bacterial removal efficiency of the filtration systems and the hydraulic retention time

The bacterial removal efficiency of each treatment system was obtained by comparing the CFU numbers obtained from the samples taken before filtration and the average CFU numbers obtained from samples taken after filtration. The log reduction in bacteria numbers was calculated using equation 1 and the percentage reduction using equation 2 (Brözel and Cloete, 1991).

Equation 1:

Log reduction = (Log_{10} bacterial count $_{\text{before filtration}}$ − Log $_{10}$ bacterial count $_{\text{after filtration}}$)

Equation 2:

$$\text{Percentage reduction} = 100 - \frac{\text{Survivor count}}{\text{Initial count}} \times 100$$

The hydraulic retention time (HRT) is a measurement of the average length of time that a soluble compound remains in a constructed bioreactor. It can be calculated by dividing the total volume of the tank by the flow rate of the influent. The total volume is measured in litres and the flow rate of influent in litres per hour. Hydraulic retention time is then expressed in hours. Equation 3 was used to calculate hydraulic retention time for each filtration system.

Equation 3:

$$\text{Hydraulic Retention Time (h)} = \frac{\text{Volume of tank (L)}}{\text{Flow rate of influent (L/h}^{-1}\text{)}}$$

5.2.6 Recovery and assay of coliphages

Coliphages were enumerated according to Baker et al. (2003). Briefly, 30 µL of chloroform (BDH AnalaR®) was added to 2 mL of an untreated rainwater sample as well as a treated rainwater sample collected from the different filtration systems. Each sample was then centrifuged at 13200 × g for five minutes. To ensure that no chloroform was transferred, 1 mL of each sample was subsequently added to 100 µL *Escherichia coli* ATCC 13706 (Microbiologics®) which had been grown to stationary phase in Luria-Bertani Broth (Merck). After each sample had been briefly vortexed, the samples were then incubated at 25˚C for five minutes. The mixture was transferred to a test tube containing 5 mL of melted top agar (7% Luria-Bertani w/v), mixed gently and poured onto a plate

containing Luria-Bertani Agar. Once the plates had set, the samples were incubated at 37˚C for 18 hours. Each assay was performed in triplicate for each sample. Rainwater samples that were spiked with *E. coli* ATCC 13706 were also analysed in the same manner and for this, 10 mL of *E. coli* DH5α that had been cultured to the stationery phase was added to 500 mL rainwater, the mixture was then allowed to stand at room temperature for approximately 6 hours before samples were collected to be analysed for the presence of coliphages.

5.2.7 Extraction of total DNA from rainwater samples

Total DNA extractions were performed for each of the 45 rainwater samples collected before and after filtration. In order to extract total genomic DNA from the rainwater samples a modified version of the boiling method was utilised (Watterworth et al., 2005). Each rainwater sample (500 mL) was filtered through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (10 kPa, 10 psi). The filters for each rainwater sample were then incubated in 2 mL Luria Bertani (LB) (Merck) broth for 5 hours at 37°C. The samples were vortexed for 15 min to detach the cells from the filters and cells were harvested from 2 mL of the cell suspension through centrifugation at 12500 × g for 10 min. The supernatant was discarded and the pellet re-suspended in 100 µL sterile MilliQ water. The re-suspended cells were then boiled at 95°C for 15 min, followed by cooling on ice for 10 min. The sample was subjected to centrifuging at 12500 × g for 5 min and the supernatant transferred to a sterile eppendorf tube. In each instance genomic DNA and total DNA was visualised on a 0.8% agarose gel stained with 0.5 µg/mL ethidium bromide. Electrophoresis was conducted at 80 volts for approximately one hour with the use of 1X Tris/Borate/EDTA (TBE) buffer (Sambrook et al., 1989).

5.2.8 Bacterial genus specific PCR reactions

Primers and PCR conditions as outlined in Table 5.2 were utilised in the current study for the identification of documented pathogenic and opportunistic bacterial pathogens. Each PCR mix was performed in a final volume of 50 µL. For the detection of *Shigella* spp., *Salmonella* spp. and *Aeromonas* spp. the PCR mix consisted of 10 µL of 5X Green GoTaq® Flexi Buffer (1X final concentration; Promega), 4 µL $MgCl_2$ (2.0 mM) (Promega), 0.5 µL of each dNTP (0.1 mM) (Thermo Scientific), 0.5 µL of the PCR primer (0.1 µM), 0.3 µL of GoTaq® Flexi DNA Polymerase (Promega) (1.5U) and 10 µL of template DNA. For *Yersinia* spp. and *Klebsiella* spp. the same PCR mix was used with the exception that 1.5 µL of the respective forward and reverse PCR primers (0.3 µM) were used. For *Pseudomonas* spp. and *Legionella* spp., again, the same

reaction mixture was used, however 2.0 and 2.5 µL of each PCR primer (0.4 and 0.5 µM, respectively) was used, respectively.

Table 5.2. Primers and PCR cycling parameters for the detection of various potential bacterial pathogens

Organism	Primer name	Primer sequence (5'-3')	PCR Cycling Parameters	Gene (Size bp)	References
Legionella spp.	JFP	AGGGTTGATAGGTTAA GAGC	5 min at 95°C; 40 cycles of 94°C for 1 min, 57°C for 1.5 min, 72°C for 1 min	Attachment invasion locus gene (386)	Jonas et al. (1995)
	JRP	CCAACAGCTAGTTGAC ATCG			
Aeromonas spp.	Aero-F	TGTCGGSGATGACATG GAYGTG	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Aerolysin (720)	Kong et al. (2002)
	Aero-R	CCAGTTCCAGTCCCAC CACTTCA			
Shigella spp.	IpaH-F	CCTTGACCGCCTTTCCG ATA	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Invasion plasmid Antigen H (606)	Kong et al. (2002)
	IpaH-R	CAGCCACCCTCTGAGG TACT			
Salmonella spp.	IpaB-F	GGACTTTTTAAAAGCG GCGG	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Invasion plasmid Antigen B (314)	Kong et al. (2002)
	IpaB-R	GCCTCTCCCAGAGCCG TCTGG			
Pseudomonas spp.	PA-GS-F	GACGGGTGAGTAATGC CTA	2 min at 95°C; 25 cycles of 94°C for 20 s, 54°C for 20 s, 72°C for 40 s	16S rRNA (618)	Spilker et al. (2004)
	PA-GS-R	CACTGGTGTTCCTTCCT ATA			
Yersinia spp.	227Fmod	GTCTGGGCTTTGCTGGT C	5 min at 95°C; 40 cycles of 94°C for 20 s, 60°C for 20 s, 72°C for 15 s	*omp*F (428 - 465)	Stenkova et al. (2008)
	669R	GCGTCGTATTTAGCAC CAACG			
Klebsiella spp.	gryA-F	CGCGTACTATACGCCA TGAACGTA	3 min at 95°C; 35 cycles of 94°C for 1 min, 50°C for 30 s, 72°C for 30 s	Gyrase A gene (383)	Brisse and Verhoef (2001)
	gyrA-C	ACCGTTGATCACTTCG GTCAGG			

The following strains were cultured as positive controls after which, genomic DNA was extracted; *Legionella pneumophila* ATCC 33152, *Shigella sonnei* ATCC 25931, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Aeromonas hydrophila* (environmental strain), *Klebsiella pneumoniae* ATCC 13385 and *Yersinia enterocolitica* ATCC 27729. All positive control organisms were obtained from Microbiologics®, unless indicated otherwise. The specificity of each primer set was confirmed by using non target DNA extracted from all the above mentioned positive controls and a negative control (sterile distilled H_2O) was also included.

All PCR products were analysed by gel electrophoresis in 1.5% agarose (Bio- Rad) containing 0.5 µg/mL ethidium bromide in 1X TBE buffer. Deoxyribonucleic acid bands were confirmed by

UV illumination and photographed using the Gel Doc 1000 documentation system (Bio-Rad). Once the size and the concentration of the PCR products had been confirmed, products of representatives of the samples were purified and concentrated using the DNA Clean & Concentrator™-5 Kit (Zymo Research) as per manufacturer's instructions. The cleaned products were then sent to the Central Analytical Facility at Stellenbosch University for sequencing. Chromatograms of each sequence were examined using FinchTV v. 1.4.0 software and were aligned using DNAman™ version 4.1.2.1 software. Sequence identification was completed using the National Centre for Biotechnology Information (NCBI) and The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest match of local similarity between isolates and the international database in GenBank, EMBL, DDBJ and PDB sequence data (Altschul et al., 1990). The sequences of representative isolates, that showed > 97% similarity (< 3% diversity) to organisms was recorded.

5.3 Results and Discussion

5.3.1 Temperature, pH, ambient temperature and rainfall

5.3.1.1 Biological Filtration: Activated Carbon and Slow Sand Filtration

The slow sand and activated carbon filtration systems were analysed during August and September, 2013. Overall an average pH of 6 was measured for all rainwater samples, collected before and after filtration. For all unfiltered and filtered rainwater samples collected for the duration of the biological filtration systems studies (both activated carbon and slow sand filtration), the temperature of the rainwater samples ranged from a low of 18˚C (02.09.2013) to the highest recorded temperature of 24˚C on two respective days (26.08.2013 and 02.09.2013). For the slow activated carbon filtration system, the average daily ambient temperature ranged from 21.3˚C (19.08.2013) (before the growth of the biofilm) to 19.7˚C (26.08.2013) on the first day of sampling to 11˚C on the last day of sampling (30.08.2013). For the slow sand filtration system, the average daily ambient temperature ranged from 21.3˚C (19.08.2013) (before the growth of the biofilm) to 21.1˚C (26.08.2013) on the first day of sampling to 14.3˚C on the last day of sampling (13.09.2013). The highest total rainfall was recorded during August 2013 (371.6 mm) which then decreased in September 2013 (177.2 mm).

5.3.1.2 Activated Carbon and Nanofibre Membrane System

Columns containing two PVA membrane layers surrounded by activated carbon were connected directly to rainwater tanks B and C during June and July, 2013. Overall an average pH of 6 was measured for all rainwater samples collected before and after filtration. The temperature of the rainwater samples ranged from a low of 14˚C (26.06.2013) to the highest recorded temperature of

20˚C (30.07.2013). From the start of the implementation of the microfiltration system, the average daily ambient temperature ranged from 15.6˚C (26.06.2013) on the first day of sampling to 20˚C on the last day of sampling (30.07.2013). The highest total rainfall was recorded during June 2013 (227.4 mm) which then decreased in July 2013 (169.6 mm).

In addition to the PVA/activated carbon filtration system, a column unit containing only two layers of the PVA membrane was also connected to tanks B and C during July and August 2013. From the start of microfiltration the average daily ambient temperature ranged from 21.3˚C (30.07.2013) on the first day of sampling to 20.2˚C (05.08.2013) on the last day of sampling. During the high rainfall period the PVA membrane system was analysed and rainfall recorded ranged from 169.6 mm (July 2013) to 371.6 mm (August 2013).

In order to determine the pore sizes of the PVA membrane filters, scanning electron microscopy (SEM) was performed (Figure 5.4) and the average pore size was calculated at 0.9 µm using the Digimizer Software version 4.25, however it should be noted that the pore size of the membrane was not uniform throughout and ranged from 0.32 µm to 1.9 µm.

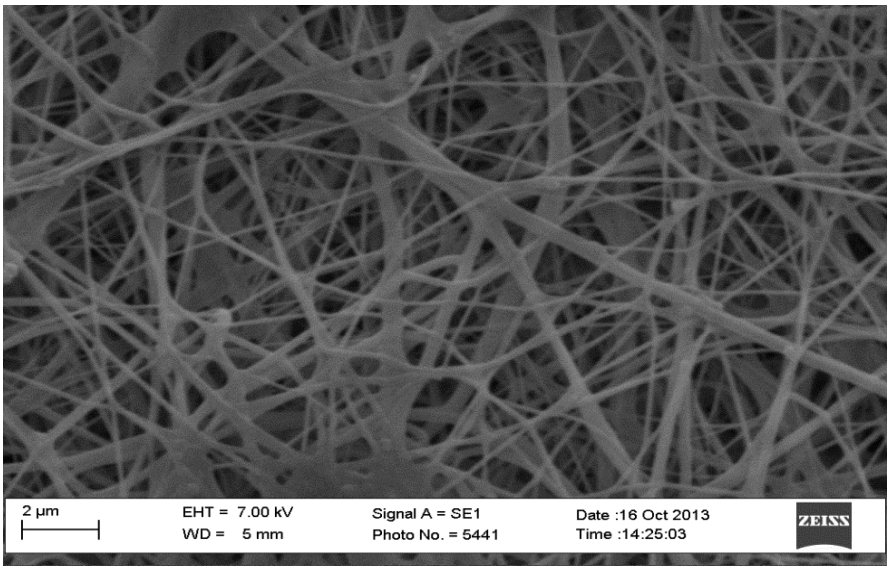


Figure 5.4. A scanning electron microscope image of a polyvinyl (alcohol) (PVA) nanofibre membrane before filtration of rainwater.

Once double layers of the PVA membrane were placed in the centre of the column with the activated carbon layered on the outer section, rainwater was allowed to pass through the activated carbon in order to remove larger contaminating particles, and then through the double layer of PVA fibres to remove smaller particles. Once rainwater samples were filtered (5 x 1 L), the PVA membrane filters were observed to be covered with particles varying in size (Figure 5.5).

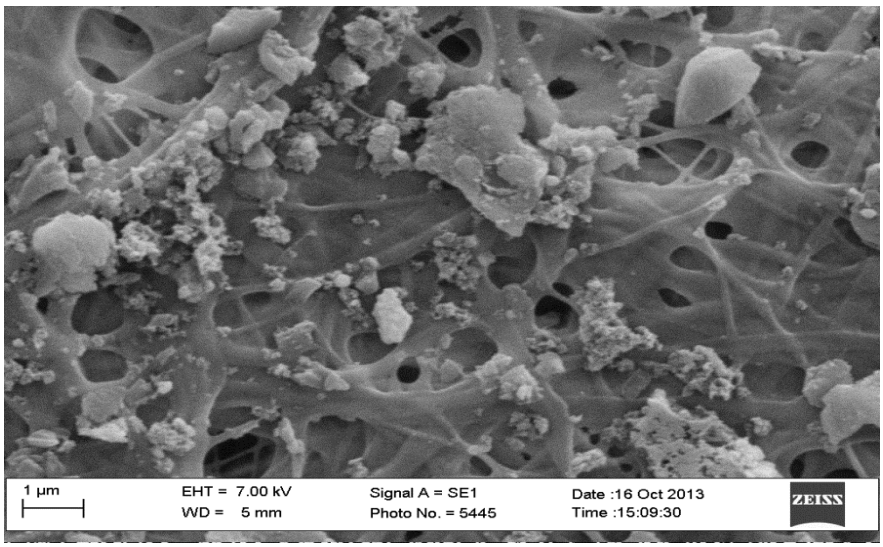


Figure 5.5. A scanning electron microscope image of a polyvinyl (alcohol) (PVA) nanofibre membrane examined after rainwater was filtered through the activated carbon and nanofibre membrane system

Cocci shaped particles, rod shaped particles, as well as other debris and possibly granules of activated carbon were observed. The average time recorded to filter 1 L of rainwater was recorded as 31.02 minutes for the first litre, 39.57 minutes for the second litre, 38.27 minutes for the third litre, 52.92 minutes for the fourth litre and 61 minutes for the fifth litre. In comparison, the filtering time for the fifth litre (61 minutes) was approximately double that of the filtering time for the first litre (31.02 minutes) as it is hypothesised that over time the membrane had become saturated and the pores had clogged (Figure 5.5).

5.3.2 Chemical analysis

5.3.2.1 Biological filtration: Activated Carbon

A slow activated carbon filtration system was connected to rainwater tanks B and C. Before the efficiency of the system was monitored a biofilm was allowed to establish for one week. The presence of cations was assessed in unfiltered and filtered rainwater samples collected on the first and the fifth day of sampling after the biofilm had formed within the slow activated carbon filtration system. All cations monitored were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of aluminium and antimony, as represented in Table 5.3.

Table 5.3. Average cation concentrations obtained from rainwater samples collected before and after filtration through the activated carbon on the respective day. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 8, average of each duplicate sample).

Metal	Before Day 1	After Day 1	Before Day 5	After Day 5	SANS 241	DWAF	ADWG	WHO
Boron as B (mg/L)	0.01	0.01	0.01	0.00	-	-	4	2.4
Calcium as Ca (mg/L)	2.76	5.07	2.40	3.02	150	200	200	-
Potassium as K (mg/L)	0.29	1.00	0.21	0.19	50	50	-	-
Magnesium as Mg (mg/L)	0.40	0.82	0.28	0.37	70	30	200	-
Sodium as Na (mg/L)	3.09	4.96	1.86	1.91	200	100	180	-
Phosphorus as P (mg/L)	0.09	0.11	0.04	0.02	-	-	-	-
Silicon as Si (mg/L)	0.20	2.58	0.17	0.41	-	-	-	-
Aluminium as Al (µg/L)	5.96	443.29	7.29	15.15	300	150	100	-
Chromium as Cr (µg/L)	0.06	0.09	<0.303	<0.303	100	50	50	50
Manganese as Mn (µg/L)	4.76	1.61	0.83	0.68	100	50	500	-
Iron as Fe (µg/L)	94.02	30.59	21.23	10.61	200	100	300	-
Cobalt as Co (µg/L)	0.06	0.10	0.02	0.09	500	-	-	-
Nickel as Ni (µg/L)	0.15	1.11	0.64	4.76	150	-	20	70
Copper as Cu (µg/L)	1.06	0.49	1.99	1.53	1000	1000	2000	2000
Zinc as Zn (µg/L)	24.94	1.96	22.46	5.73	5000	3000	3000	-
Arsenic as As (µg/L)	0.24	7.41	0.23	0.77	10	10	10	10
Selenium as Se (µg/L)	2.36	1.18	1.88	0.68	20	20	10	40
Strontium as Sr (mg/L)	21.76	160.29	18.14	52.56	-	-	-	-
Molybdenum as Mo (µg/L)	<0.000	<0.00	0.03	0.06	-	-	50	-
Cadmium as Cd (µg/L)	0.02	0.01	<0.019	<0.019	5	5	2	3
Tin as Sn (µg/L)	0.01	0.02	<0.016	<0.016	-	-	-	-
Antimony as Sb µg/L	0.08	10.57	0.08	0.24	-	-	3	20
Barium as Ba (µg/L)	21.06	20.37	17.20	31.76	-	-	2000	700
Mercury as Hg (µg/L)	0.01	0.03	0.02	<0.021	1	1	1	6
Lead as Pb (µg/L)	0.18	0.17	0.13	0.05	20	10	10	10

The average concentration of aluminium observed for the filtered rainwater samples (443.29 µg/L) collected on day one of sampling exceeded the recommended guidelines of 300 µg/L stipulated by SANS 241 (2005), 150 µg/L as stipulated by DWAF (1996) and 100 µg/L as stipulated by the AWDG (NHMRC and NRMMC, 2011). In addition, the average concentration of antimony in the rainwater sample collected on day one after filtration through the activated carbon system, exceeded the AWDG (NHMRC and NRMMC, 2011) guideline of 3 µg/L, with an average concentration of 10.57 µg/L observed. However, the concentrations of aluminium and antimony in the remaining unfiltered and filtered rainwater samples collected on day 5 were within the respective guidelines.

Although aluminium has been associated with Alzheimer's disease, Parkinsonism dementia and amyotrophic lateral sclerosis, it has been concluded that there is insufficient information to link these diseases with the consumption of aluminium through drinking water sources (DWAF, 1996; NHMRC and NRMMC, 2011). In studies performed on rats, the consumption of antimony was also linked to fertility and it was demonstrated that antimony accumulates in the heart, spleen, liver and kidney (NHMRC and NRMMC, 2011).

While it was noted that all other cations detected were within the respective guidelines, the significant variations in the concentrations of the cations as well as aluminium and antimony (that were not within guidelines) will be discussed. The concentration of silicon, aluminium, arsenic, antimony and strontium present in the filtered rainwater samples collected on day one after the formation of the biofilm increased significantly (more than a 10 fold increase) from an average of 0.2 µg/L (unfiltered) to an average of 2.58 µg/L (filtered), from an average of 5.96 µg/L (unfiltered) to an average of 443.29 µg/L (filtered), from an average of 0.24 µg/L (unfiltered) to an average of 7.41 µg/L (filtered), from an average of 0.08 µg/L (unfiltered) to an average of 10.57 µg/L (filtered), and from an average of 21.76 µg/L (unfiltered) to an average of 160.29 µg/L (filtered) respectively. In contrast, in the same rainwater samples the concentrations of manganese, iron, copper and zinc were observed to have decreased on day one after filtration through the activated carbon from an average of 4.76 µg/L (unfiltered) to an average of 1.61 µg/L (filtered), from an average of 94.02 µg/L (unfiltered) to an average of 30.59 µg/L (filtered), from an average of 1.06 µg/L (unfiltered) to an average of 0.49 µg/L (filtered) and from an average of 24.94 µg/L (unfiltered) to an average of 1.96 µg/L (filtered), respectively. On the fifth day after the formation of the biofilm, no significant increases were observed for any of the cations present in the rainwater samples, however potassium, phosphorous, manganese, iron, copper, zinc, selenium and lead exhibited a negligible decrease after filtration through the activated carbon system.

The raw materials used in the manufacturing of commercially available activated carbon contains ppm levels of arsenic and antimony and have been shown to leach small fractions of these elements when in direct contact with water (Vaughn and Distefano, 2013). This could account for the increased levels of antimony and arsenic observed in the rainwater samples that have been filtered through the activated carbon. Moreover ash, used in the manufacturing of activated carbon, contains aluminium and silicon (Block and Dams, 2010). Therefore aluminium and silicon could also have leached from the activated carbon particles into the rainwater during the filtration process.

All anions present in the unfiltered and filtered rainwater samples collected on day one and day five after the formation of the biofilm on activated carbon were within drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) and are represented in Table 5.4.

Table 5.4. Average anion concentrations obtained from rainwater samples collected before and after filtration through the activated carbon on the respective days. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 8, average of each duplicate sample).

Anions (mg/L)	Before Day 1	After Day 1	Before Day 5	After Day 5	SANS 241	DWAF	ADWG	WHO
Sulphate as SO_4	1.15	1.7	1.4	1.55	200	100	250	-
Chloride as Cl^-	6.65	2.9	6.55	4.9	400	200	250	-
Nitrate and Nitrite as NO_3 and NO_2	0.2	0.1	0.05	0.05	10	6	50	50
Phosphate as PO_4	0.095	0.085	0.025	0.025	-	-	-	-
Fluoride as F	<0.01	<0.01	0.1	0.1	1	1	1.5	1.5

On the first day of the trial chloride and nitrite and nitrate concentrations decreased from an average of 6.65 mg/L (unfiltered) to an average of 2.9 mg/L (filtered) and from an average of 0.2 mg/L (unfiltered) to an average of 0.1 mg/L (filtered), respectively. On the fifth day of filtration only the concentration of chloride was shown to have decreased from an average of 6.55 mg/L (unfiltered) to an average of 4.9 mg/L (filtered).

The formation of a biofilm layer on granular activated carbon (GAC) is referred to as biological activated carbon (BAC) and has gained interest as a water treatment technology. Biological activated carbon has been shown to remove chemical pollutants, disinfection by-product precursors and organic matter (Simpson, 2008). In this study the biofilm was allowed to establish for a week before the rainwater was filtered through the activated carbon. The efficiency of this system could therefore be increased by lengthening the time of biofilm growth before using the system for the filtering of rainwater.

5.3.2.2 Biological Filtration: Slow Sand Filtration

A slow sand filtration system was installed on rainwater tanks B and C. Before the efficiency of the system was monitored a biofilm was allowed to establish for two weeks. The presence of cations was assessed in unfiltered and filtered rainwater samples collected on the first and the twelfth day after the formation of the biofilm within the slow sand filtration system. All cations present in the rainwater samples collected before and after slow sand filtration were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of aluminium, manganese and iron as represented in Table 5.5.

The aluminium concentration of the filtered rainwater sample (average 1601.43 µg/L) collected on day one exceeded the recommended guidelines as stipulated by SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011). However, the concentrations of aluminium in both unfiltered rainwater samples and the filtered rainwater sample collected on day twelve were within the recommended guidelines. The concentration of manganese in the filtered rainwater sample collected on day one had an average concentration of 53.45 µg/L which exceeded the recommended guideline of 50 µg/L stipulated by DWAF (1996). However, the concentrations of manganese in both unfiltered rainwater samples and the filtered rainwater sample collected on day twelve also adhered to recommended guidelines as stipulated by SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011).

Only the unfiltered rainwater sample, collected on day one, adhered to the recommended guidelines for iron as stipulated by SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) with an average concentration of 23.04 µg/L observed. However, the average concentrations of iron in the unfiltered rainwater samples collected on day twelve (143.99 µg/L) and the filtered rainwater sample collected on day twelve (108.31 µg/L) were not within the recommended DWAF (1996) guideline of 100 µg/L. In addition, the concentration of iron in the filtered rainwater sample collected on day one (4083.45 µg/L) was not within the guidelines as stipulated by SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011).

As mentioned previously (Section 5.3.2.1), aluminium present in drinking water has been linked to certain neurodegenerative diseases. Manganese is however, considered to have very low health risks if present in water, but can have aesthetic and taste effects if present at concentrations exceeding the respective guidelines (DWAF, 1996; NHMRC and NRMMC, 2011).

Table 5.5. Average cation concentrations obtained from rainwater samples collected before and after filtration through the slow sand filter on day one and twelve, respectively. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 8, average of each duplicate sample).

Metal	Before Day 1	After Day 1	Before Day 12	After Day 12	SANS 241	DWAF	ADWG	WHO
Boron as B (mg/L)	0.01	0.02	0.01	0.01	-	-	4	2.4
Calcium as Ca (mg/L)	2.95	5.32	2.87	3.04	150	200	200	-
Potassium as K (mg/L)	0.20	0.51	0.27	0.33	50	50	-	-
Magnesium as Mg (mg/L)	0.33	0.73	0.38	0.44	70	30	200	-
Sodium as Na (mg/L)	2.33	3.63	2.67	2.77	200	100	180	-
Phosphorus as P (mg/L)	0.01	0.01	0.02	0.03	-	-	-	-
Silicon as Si (mg/L)	0.21	0.99	0.30	0.54	-	-	-	-
Aluminium as Al (µg/L)	6.17	1601.43	5.42	6.73	300	150	100	-
Chromium as Cr (µg/L)	<0.303	1.98	<0.303	<0.303	100	50	50	50
Manganese as Mn (µg/L)	1.39	53.45	2.92	2.19	100	50	500	-
Iron as Fe (µg/L)	23.04	4083.45	143.99	108.31	200	100	300	-
Cobalt as Co (µg/L)	0.15	1.92	0.08	0.10	500	-	-	-
Nickel as Ni (µg/L)	11.77	16.74	2.62	5.48	150	-	20	70
Copper as Cu (µg/L)	11.61	7.31	6.19	7.99	1000	1000	2000	2000
Zinc as Zn (µg/L)	21.95	67.69	20.96	24.05	5000	3000	3000	-
Arsenic as As (µg/L)	0.26	0.61	0.31	0.69	10	10	10	10
Selenium as Se (µg/L)	1.02	1.54	0.85	1.20	20	20	10	40
Strontium as Sr (mg/L)	20.35	28.96	24.42	25.68	-	-	-	-
Molybdenum as Mo (µg/L)	0.06	0.03	0.03	0.09	-	-	50	-
Cadmium as Cd (µg/L)	<0.019	0.09	0.03	<0.019	5	5	2	3
Tin as Sn (µg/L)	<0.016	0.03	<0.016	<0.016	-	-	-	-
Antimony as Sb µg/L	0.10	0.12	0.12	0.23	-	-	3	20
Barium as Ba (µg/L)	20.78	3.86	32.12	19.15	-	-	2000	700
Mercury as Hg (µg/L)	0.06	<0.021	0.04	0.04	1	1	1	6
Lead as Pb (µg/L)	0.15	0.24	0.16	0.10	20	10	10	10

The noteworthy changes in the concentrations of the cations present in the rainwater samples before and after slow sand filtration will be discussed first. A significant increase (more than 10 fold increase) in the concentrations of aluminium, manganese, iron and cobalt was observed after slow sand filtration on day one with concentrations before and after filtration ranging from an average of 6.17 µg/L (unfiltered) to an average of 1601.43 µg/L (filtered), from an average of 1.39 µg/L (unfiltered) to an average of 53.45 µg/L (filtered), from an average of 23.04 µg/L (unfiltered) to an average of 4083.45 µg/L (filtered), and from an average of 0.15 µg/L (unfiltered) to an average of 1.92 µg/L (filtered), respectively. Copper, molybdedum, mercury and barium were the only cations that decreased after slow sand filtration on day one with concentrations before and after filtration ranging from an average of 11.61 µg/L (unfiltered) to an average of 7.31 µg/L (filtered), from an average of 0.06 µg/L (unfiltered) to an average of 0.03 µg/L (filtered), from an average of 0.06 µg/L (unfiltered) to an average of <0.021 µg/L (filtered) and from an average of 20.78 µg/L (unfiltered) to an average of 3.86 µg/L, respectively. All the other cations detected after slow sand filtration on day one also increased, however the increase was negligible (Table 5.5).

The concentrations of manganese, iron, barium, lead and cadmium decreased after slow sand filtration on day twelve with concentrations before and after filtration ranging from an average of 2.92 µg/L (unfiltered) to an average of 2.19 µg/L (filtered), from an average of 143.99 µg/L (unfiltered) to an average of 108.31 µg/L (filtered), from an average of 32.12 µg/L (unfiltered) to an average of 19.15 µg/L (filtered), from an average of 0.16 µg/L (unfiltered) to an average of 0.10 µg/L (filtered), and from an average of 0.03 µg/L (unfiltered) to an average of < 0.019 µg/L (filtered), respectively. All the cations detected after slow sand filtration on day twelve increased, however the observed increases were not significant (Table 5.5).

A study by Mwabi et al. (2011) investigated the efficiency of a bucket filter (BF), consisting of a layer of gravel and sand, and a biosand filter (BSF), consisting of a layer of zeolites, sand and a biological layer. The BF and BSF were shown, in both cases, to reduce the concentration of iron before filtration from 0.03 mg/L to < 0.01 mg/L after filtration. The concentration of iron present in the rainwater, in this study, was shown to increase after the first day of the study significantly (more than a 10 fold increase) from an average of 23.04 µg/L (unfiltered) to an average of 4083.45 µg/L (filtered), and to decrease after filtration on day twelve from an average of 143.99 µg/L (unfiltered) to an average of 108.31 µg/L (filtered). It could be hypothesised that in the current study a longer time period was required to form an effective biological layer as, rainwater, is generally less contaminated than wastewater, which was used in the study by Mwabi et al. (2011). Mwabi et al. (2011) also incorporated a layer of zeolites into the biosand filter which is known to have high removal efficiencies for chemical contaminants.

All anions present in the rainwater samples collected before and after filtration through the slow sand system were within drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) and are represented in Table 5.6.

Table 5.6. Average anion concentrations obtained from rainwater samples collected before and after filtration through the slow sand filter on day one and twelve, respectively. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 8, average of each duplicate sample).

Anions (mg/L)	Before Day 1	After Day 1	Before Day 12	After Day 12	SANS 241	DWAF	ADWG	WHO
Sulphate as SO_4	1.1	41.5	1.45	4.15	200	100	250	-
Chloride as Cl^-	5.45	5.65	5.95	5.1	400	200	250	-
Fluoride as F	<0.1	<0.1	<0.1	<0.1	1	1	1.5	1.5

A significant increase in the concentration of sulphate on day one after slow sand filtration was observed with concentrations before and after filtration ranging from an average of 1.1 mg/L (unfiltered) to an average of 41.5 mg/L (filtered). The increase in sulphate concentrations after slow sand filtration on day twelve was however, less than on the first day with average concentrations ranging from 1.45 mg/L (unfiltered) to an average of 4.15 mg/L (filtered). The concentration of chloride was observed to have increased on day one with concentrations before and after filtration ranging from an average of 5.45 mg/L (unfiltered) to an average of 5.65 mg/L (filtered) and then on day 12 from an average of 5.95 mg/L (unfiltered) decreased to an average of 5.1 mg/L (filtered), respectively. As previously noted the bacterial population within the biofilm layer degrades organic matter present in water samples into carbon dioxide, and inorganic salts such as sulphates. Therefore the presence of sulphur oxidizing bacteria within a biofilm layer that might have developed on the sand medium could have contributed to the increased concentrations of sulphate in the filtered rainwater samples (WHO, 1974). Previous studies have shown that inorganic compounds can accumulate in the biofilm of the slow sand filter (Hijnen et al., 2004). This could thus explain the decrease in the concentration of chloride present in the rainwater after filtration through the slow sand filter.

5.3.2.3 Activated carbon and nanofibre membrane system

All cations monitored in the rainwater samples collected before and after (first and fifth litre only analysed) filtration through the activated carbon and nanofibre membrane were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) as represented in Table 5.7.

However, the concentrations of copper, antimony, lead, aluminium, arsenic and strontium increased in the first litre of rainwater filtered through the activated carbon and nanofibre membrane system from an average of 0.89 µg/L (unfiltered) to an average of 33.40 µg/L (filtered), from an average of 0.14 µg/L (unfiltered) to an average of 1.66 µg/L (filtered) from an average of 0.02 µg/L (unfiltered) to an average of 5.28 µg/L (filtered), and from an average of 9.42 µg/L (unfiltered) to an average of 54.49 µg/L (filtered), from an average of 0.34 µg/L (unfiltered) to an average of 3.28 µg/L (filtered) and from an average of 24.69 µg/L (unfiltered) to an average of 61.31 µg/L (filtered), respectively. In addition, in the fifth litre of filtered rainwater the concentrations of copper, antimony, lead, aluminium, nickel, arsenic and strontium increased from an average of 0.89 µg/L (unfiltered) to an average of 1.35 µg/L (filtered), from an average of 0.14 µg/L (unfiltered) to an average of 1.75 µg/L (filtered), from an average of 0.02 µg/L (unfiltered) to an average of 0.32 µg/L (filtered), from an average of 9.42 µg/L (unfiltered) to an average of 65.63 µg/L (filtered), from an average of 0.32 µg/L (unfiltered) to an average of 1.22 µg/L (filtered), from an average of 0.34 µg/L (unfiltered) to an average of 2.36 µg/L (filtered) and from an average of 24.69 µg/L (unfiltered) to an average of 81.07 µg/L (filtered) respectively.

Table 5.7. Cation concentrations obtained from rainwater samples collected before filtration through activated carbon and the nanofibre layers and well as the cation concentrations present in the 1st and 5th litre of filtered rainwater. Cation concentrations are compared to the respective drinking water guidelines (n = 3).

Metal	Before activated carbon and nanofibres	After activated carbon and nanofibres (1ST L)	After activated carbon and nanofibres (5th L)	SANS 241	DWAF	ADWG	WHO
Calcium as Ca (mg/L)	3.53	4.15	4.49	150	200	200	-
Potassium as K (mg/L)	0.22	0.59	0.28	50	50	-	-
Magnesium as Mg (mg/L)	0.43	0.45	0.60	70	30	200	-
Sodium as Na (mg/L)	3.00	2.81	3.15	200	100	180	-
Phosphorus as P (mg/L)	0.02	1.13	0.03	-	-	-	-
Silicon as Si (mg/L)	0.32	0.85	1.13	-	-	-	-
Aluminium as Al (µg/L)	9.42	54.49	65.63	300	150	100	-
Chromium as Cr (µg/L)	0.21	0.11	0.04	100	50	50	50
Manganese as Mn (µg/L)	2.78	1.92	3.35	100	50	500	-
Iron as Fe (µg/L)	90.20	21.83	5.81	200	100	300	-
Cobalt as Co (µg/L)	0.04	0.05	0.07	500	-	-	-
Nickel as Ni (µg/L)	0.32	0.32	1.22	150	-	20	70
Copper as Cu (µg/L)	0.89	33.40	1.35	1000	1000	2000	2000
Zinc as Zn (µg/L)	14.95	17.88	13.01	5000	3000	3000	-
Arsenic as As (µg/L)	0.34	3.28	2.36	10	10	10	10
Selenium as Se (µg/L)	1.38	1.04	1.05	20	20	10	40
Strontium as Sr (mg/L)	24.69	61.31	81.07	-	-	-	-
Molybdenum as Mo (µg/L)	<0.000	0.05	0.01	-	-	50	-
Cadmium as Cd (µg/L)	0.01	0.01	0.01	5	5	2	3
Tin as Sn (µg/L)	<0.000	0.03	0.00	-	-	-	-
Antimony as Sb µg/L	0.14	1.66	1.75	-	-	3	20
Barium as Ba (µg/L)	17.11	16.31	28.38	-	-	2000	700
Mercury as Hg (µg/L)	0.11	0.12	0.12	1	1	1	6
Lead as Pb (µg/L)	0.02	5.28	0.32	20	10	10	10

All anions present in the rainwater samples collected before and after (first and fifth litre only analysed) filtration through the activated carbon and nanofibre membrane were within drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) and are represented in Table 5.8.

Table 5.8. Anion concentrations obtained from rainwater collected before filtration through activated carbon and the nanofibre layers and well as the cation concentrations present in the 1st and 5th litre of rainwater filtered through the activated carbon and nanofibre layer. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 3).

Anions (mg/L)	**Before activated carbon and nanofibres**	**After activated carbon and nanofibres (1ST L)**	**After activated carbon and nanofibres (5th L)**	**SANS 241**	**DWAF**	**ADWG**	**WHO**
Sulphate as SO_4	1.5	14	1	200	100	250	-
Chloride as Cl^-	5.9	2.6	3.2	400	200	250	-
Nitrate and Nitrite as NO_3 and NO_2	0.3	0.2	0.2	10	6	50	50
Phosphate as PO_4	ND	<0.05	ND	-	-	-	-
Fluoride as F	<0.1	<0.1	<0.1	1	1	1.5	1.5

ND Not Detected

5.3.3 The Bacterial Removal Efficiency of the Filtration Systems

5.3.3.1 Biological Filtration: Activated Carbon

Total coliforms were enumerated before and after biofilm formation on the activated carbon in a slow filtration system (Figure 5.6). Before a biofilm was allowed to establish for one week, average total coliform counts were determined for unfiltered (6.2×10^2 CFU/100 mL) and initial filtered (4.5×10^2 CFU/100 mL) rainwater samples. Although no log reduction was observed, a reduction of 27% was recorded for the average total coliforms after the once off filtration through the slow activated carbon system prior to the formation of a biofilm.

Once the biofilm was established in the slow activated carbon filtration system total coliforms detected in the unfiltered rainwater samples ranged from an average of 3.9×10^2 CFU/100 mL recorded for day one to an average of 4.5×10^2 CFU/100 mL recorded on day five (Figure 5.6). The average total coliforms in filtered rainwater samples ranged from 4.5×10^2 CFU/100 mL on day one to 2.7×10^2 CFU/100 mL on day five. On day one a 13.7% increase in average total coliform numbers was observed for the filtered (4.5×10^2 CFU/100 mL) rainwater samples in comparison to the unfiltered (3.9×10^2 CFU/100 mL) rainwater samples. However, on day two no change was observed between total coliforms detected in the unfiltered rainwater samples in comparison to the

filtered rainwater samples. In contrast, on days three, four and five, reductions of 39%, 46% and 41% were observed in average total coliform numbers, respectively, for the rainwater samples filtered through the activated carbon filtrations system in comparison to the unfiltered rainwater samples.

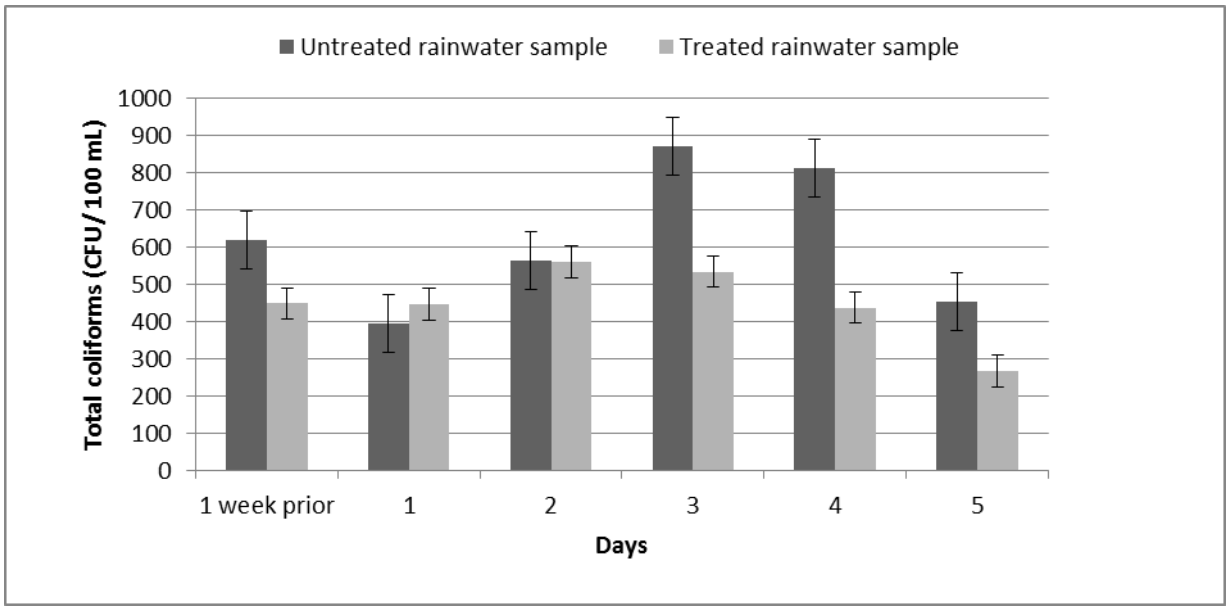


Figure 5.6. Total coliforms enumerated before the growth of a biofilm (one week prior) on activated carbon used in a slow filtration system followed by five days of monitoring the efficacy of the slow activated carbon filtration system.

No significant correlation could be established between total coliforms detected in filtered rainwater samples and an increase over time for the five day period ($R = -0.7$, $p > 0.05$). Therefore it is hypothesised that fluctuations in the filtered and unfiltered rainwater total coliform results influenced the efficiency of the activated carbon system over time. In addition, although reductions in total coliforms numbers were observed for filtered rainwater samples in comparison to unfiltered rainwater samples for days three till five, the reduction was not significant ($p = 0.16$).

Escherichia coli were enumerated before and after biofilm formation on activated carbon in a slow filtration system (Figure 5.7). Before a biofilm was allowed to establish for one week, average *E. coli* counts were determined for unfiltered (9 CFU/100 mL) and initial filtered (3 CFU/100 mL) rainwater samples. Although no log reduction was observed, a reduction of 65% in the average *E. coli* numbers was recorded after slow activated carbon filtration prior to the formation of a biofilm.

After a biofilm had established in the slow activated carbon filtration system, average *E. coli* numbers detected in the unfiltered rainwater samples ranged from 4.9×10^1 CFU/100 mL on day one to 2.1×10^1 CFU/100 mL on day five (Figure 5.7). *Escherichia coli* detected in filtered

rainwater samples ranged from 6.3 CFU/100 mL on day one to 2.3 CFU/100 mL on day five of slow activated carbon filtration. On days one, two, three, four and five reductions of 87%, 64%, 85%, 22% and 89% were observed for average *E. coli* numbers for filtered rainwater samples in comparison to unfiltered rainwater samples, respectively.

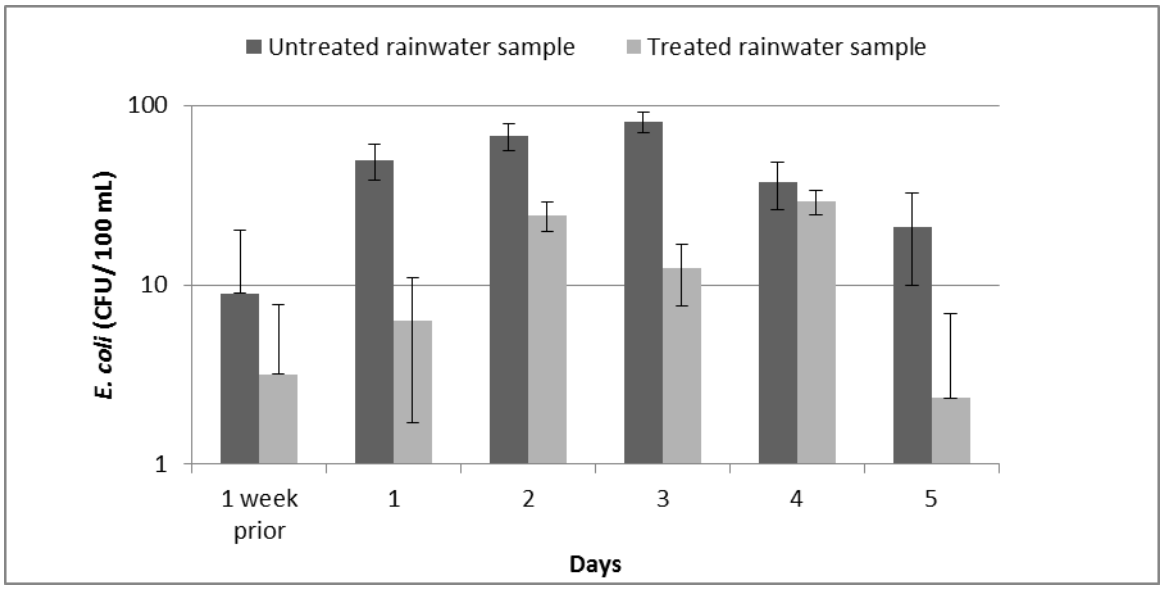


Figure 5.7. *Escherichia coli* numbers enumerated before the growth of a biofilm (one week prior) on activated carbon used in a slow filtration system followed by five days of monitoring the efficacy of the slow activated carbon filtration system.

No significant correlation could be established between *E. coli* numbers detected in filtered rainwater samples and an increase over time for the five day period ($R = -0.1$, $p > 0.05$). As for the removal of total coliforms, fluctuations in the filtered and unfiltered rainwater *E. coli* counts influenced the efficiency of the activated carbon system over time. However a significant decrease ($p < 0.003$) in *E. coli* numbers was recorded for filtered rainwater samples compared to unfiltered rainwater samples.

Heterotrophic plate counts were enumerated before and after biofilm formation on activated carbon in a slow filtration system (Figure 5.8). Before a biofilm was allowed to establish for one week, average heterotrophic bacterial counts were determined for unfiltered (1.3×10^3 CFU/ mL) and initial filtered (1.29×10^3 CFU/ mL) rainwater samples. No significant reduction was however, observed in the average HPC counts obtained after slow activated carbon filtration prior to the formation of a biofilm.

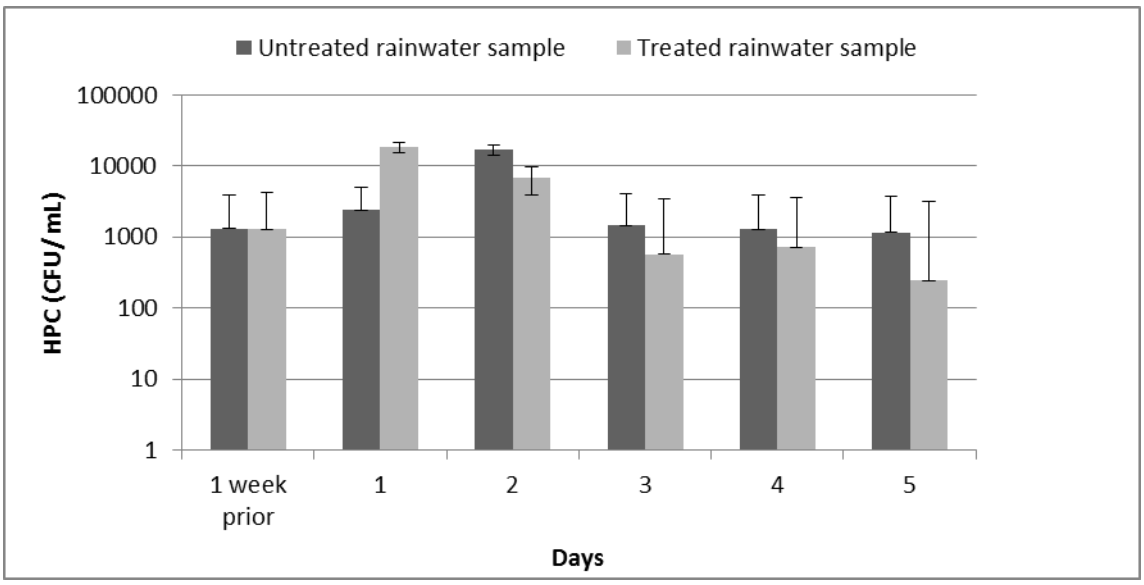


Figure 5.8. The HPC numbers enumerated before the growth of a biofilm (one week prior) on activated carbon used in a slow filtration system followed by five days of monitoring the efficacy of the slow activated carbon filtration system.

After a biofilm had established in the slow activated carbon system, average HPC numbers detected in the unfiltered rainwater samples ranged from 2.4×10^3 CFU/mL on day one to 1.2×10^3 CFU/mL on day five. Heterotrophic plate count bacteria detected in filtered rainwater samples ranged from 1.9×10^4 CFU/mL on day one to 2.3×10^2 CFU/mL on day five of slow activated carbon filtration. On day one a 7 -fold increase in average HPC numbers was observed for filtered (1.9×10^4 CFU/ mL) rainwater samples compared to the unfiltered (2.4×10^3 CFU/ mL) rainwater samples. However, on days two, three, four and five, reductions of 59%, 60%, 45% and 79% were observed for the average HPC counts for filtered rainwater samples compared to unfiltered rainwater samples, respectively.

A significant negative correlation was established between HPC detected in filtered rainwater samples and an increase over time for the five day period ($R = - 0.9$, $p < 0.05$). It is thus hypothesised, that with the exception of day one where heterotrophic bacteria could have sloughed off the biofilm, more heterotrophic bacteria were removed by the activated carbon biofilm layer over time. However, although reductions in HPC numbers were observed for filtered rainwater samples in comparison to unfiltered rainwater samples for days two till five, the overall reduction was not significant ($p = 0.84$).

According to guidelines stipulated by SANS 241 (2005), the Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011) and the Department of Water Affairs and Forestry (DWAF, 1996), no *E. coli* should be present in a water sample. No guideline values for total

coliforms and HPC have been stipulated by the SANS 241 (2005) and the ADWG (NHMRC and NRMMC, 2011), however, DWAF (1996) has stipulated that total coliform and HPC should not exceed 5 CFU/100 mL and 100 CFU/mL, respectively.

Escherichia coli values for all unfiltered and filtered rainwater samples using the slow activated carbon filtration systems did not adhere to the guidelines stipulated by SANS 241 (2005), the ADWG (NHMRC and NRMMC, 2011) and DWAF (1996). Moreover, total coliform and HPC values for all unfiltered and filtered rainwater samples using the slow activated carbon filtration systems did also not adhere to the guidelines stipulated by DWAF (1996).

The hydraulic retention time for the activated carbon filtration system was calculated as follows:

$$HRT = \frac{15\ L}{\frac{0.5\ L}{5\ min}} = \frac{15\ L}{6\ L.h^{-1}} = 2.5\ h$$

A soluble compound would thus remain in the constructed slow activated carbon filter for approximately 2.5 hours.

Before the biofilm was allowed to establish in the slow activated carbon filtration system, total coliforms, *E. coli* and HPC numbers were reduced in the rainwater samples by 27%, 65% and 2% after an initial filtration through the activated carbon system, respectively. Once the biofilm had been established for a week the average total coliforms, *E. coli* and HPC for the whole sampling period (days one to five) were reduced by 27%, 71%, and no reduction, after filtration of rainwater through the activated carbon system, respectively. Therefore, no significant reduction ($p = 0.93$) could be established for total coliforms, *E. coli* and HPC percentages recorded before and after the growth of the biofilm in the slow activated carbon system. It could be hypothesised that the ineffectiveness of the slow activated carbon filtration system in removing indicator bacteria could be due to the growth of an immature biofilm, as the biofilm was only established for approximately one week. Many studies have shown that biological activated carbon, which is granular activated carbon on which bacteria have been immobilised, requires an extended time period before adequate amounts of biomass are attained (LeChevallier et al., 1984; Gao et al., 2010; Zhang et al., 2013). Many studies have also indicated that biological activated carbon has proven effective in removing for example dissolved organic matter and ammonium (Andersson et al., 2001; Tian et al., 2009). Lately, a bio–enhanced approach has been shown to be more effective in removing pollutants (Gao et al., 2010; Zhang et al., 2013). This approach entails adding a consortium of competent microorganisms, for example Zhang et al. (2013) added bacteria that were able to biodegrade high concentrations of total organic carbon and exhibited high dehydrogenase activity. These bacteria included *Pseudomonas putita*, *Pseudomonas pertucinigena*, *Pseudomonas balearica* and *Bacillus*

subtilis, which resulted in a bio-enhanced activated carbon (BEAC). To date no studies have indicated the efficiency of granular activated carbon filtration systems in removing indicator bacteria from rainwater. The focus is generally on the functionality of the biological biomass found within the granular activated carbon particles and the ability of the activated carbon systems to remove chemical particle and other contaminants such as total organic carbon (TOC) (Lehtola et al., 2002; Mohan and Pittman, 2006; Gibert et al., 2012). However, some studies have shown that cells attached to activated carbon particles show resistance to disinfection with chlorine. LeChevallier et al. (1984) showed that disinfecting HPC bacteria, coliform organisms and other pathogenic microorganisms attached to the activated carbon with chlorine (2.0 mg/L) for one hour, showed no significant decrease in viable counts, with similar results observed when the bacteria were washed from the activated carbon.

5.3.3.2 Slow Sand Filtration

Total coliforms were enumerated before and after biofilm formation on sand in a slow filtration system (Figure 5.9). Before a biofilm was allowed to establish for two weeks, average total coliforms were determined from unfiltered (6.9×10^2 CFU/100 mL) and initial filtered rainwater samples, with no total coliforms recovered from the filtered rainwater samples and a three log reduction thus recorded for the average total coliforms after slow sand filtration prior to the formation of a biofilm.

After a biofilm had established in the slow sand filtration system, average total coliform numbers detected in the unfiltered rainwater samples ranged from 1.5×10^2 CFU/100 mL on day one to 1.38×10^3 CFU/100 mL on day twelve. Total coliforms detected in filtered rainwater samples ranged from 7.7×10^1 CFU/100 mL on day one to 1.37×10^3 CFU/100 mL on day twelve of the slow sand filtration system. On days one, three, five, eight, ten and twelve, reductions of 49.8%, 72.9%, 82.8%, 67.4%, 89.4% and 0.4% were thus observed in average total coliform numbers, respectively.

Overall, a significant positive correlation ($R = 0.94$, $p < 0.05$) was established between average total coliforms recorded for the filtered rainwater samples and an increase in time (days one to twelve). This could be due to an increase observed in total coliforms for the unfiltered rainwater samples over time. However, reductions in total coliform numbers observed for filtered rainwater samples for days one to twelve were not significant ($p = 0.084$).

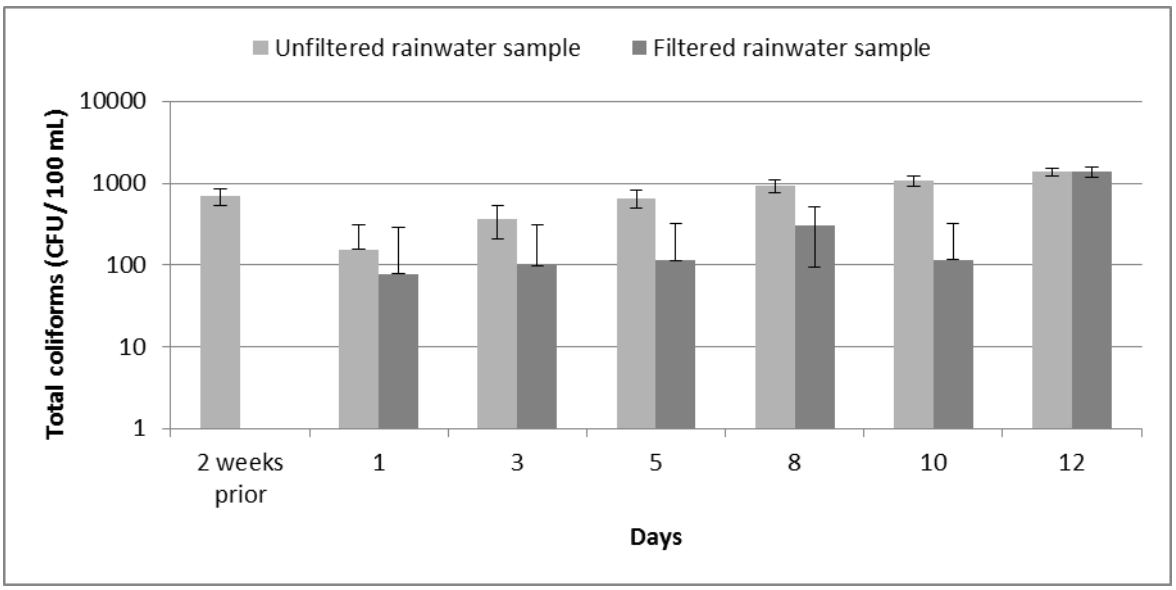


Figure 5.9. Total coliforms enumerated before the growth of a biofilm (two weeks prior) on sand used in a slow filtration system followed by six days (every second day) of monitoring the efficacy of the slow sand filtration system.

During the establishment of a biofilm (two weeks), average *E. coli* numbers were determined for unfiltered (9 CFU/100 mL) and initial filtered rainwater samples, with no *E. coli* recovered for the filtered rainwater samples and a reduction in average *E. coli* numbers recorded after slow sand prior to the formation of a biofilm (Figure 5.10).

After a biofilm had established in the slow sand filtration system, average *E. coli* numbers detected in the unfiltered rainwater samples ranged from 2 CFU/100 mL on day one to 7 CFU/100 mL on day twelve. *Escherichia coli* detected in filtered rainwater samples ranged from no *E. coli* (0.2 CFU/100 mL) detected on day one to 1.2×10^1 CFU/100 mL on day twelve of the slow sand filtration system. On days one, three, five and eight reductions of 91.7%, 71.4%, 66.7% and 100% were observed in average *E. coli* numbers for filtered rainwater samples compared to unfiltered rainwater samples, respectively. On days ten and twelve the number of *E. coli* however increased significantly ($p < 0.05$) in comparison to the unfiltered *E. coli* count obtained. No significant corresponding increases in total coliforms and heterotrophic bacterial counts were however, observed on the same sampling days. It has also previously been noted that *E. coli* is regarded as the most specific indicator of faecal contamination. The filtering system could thus have possibly been contaminated with faecal matter passing through the tank and lodging in the sand particles.

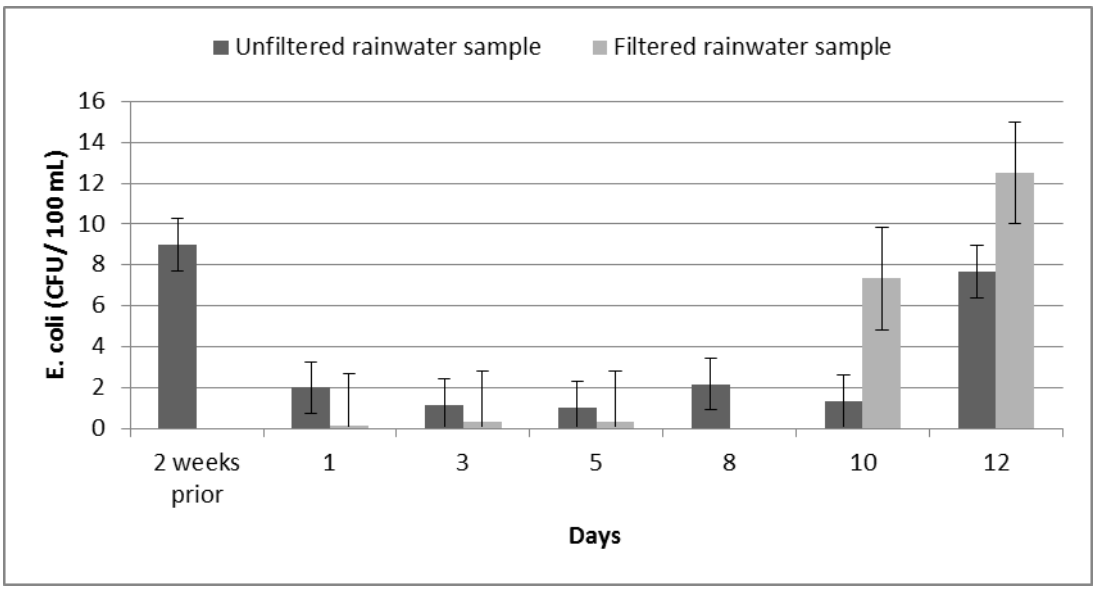


Figure 5.10. *Escherichia coli* numbers enumerated before the growth of a biofilm (two weeks prior) on sand used in a slow filtration system followed by six days (every second day) of monitoring the efficacy of the slow sand filtration system.

No significant correlation ($R = 0.64$, $p > 0.05$) was established between average *E. coli* numbers recorded for the filtered rainwater samples and an increase in time (days one to twelve). Therefore it is hypothesised that fluctuations in the filtered and unfiltered rainwater *E. coli* results influenced the efficiency of the sand filtration system over time. Although reductions in *E. coli* numbers were observed for filtered rainwater samples for days one, three, five and eight, the reduction was not significant ($p = 0.82$).

Heterotrophic plate counts enumerated before and after biofilm formation on silica sand in a slow filtration system are indicated in Figure 5.11. Before a biofilm was allowed to establish for two weeks, average HPC numbers were determined from unfiltered (1.3×10^3 CFU/ mL) and initial filtered (1.3×10^1 CFU/ mL) rainwater samples with a two log reduction observed.

After a biofilm had established in the slow sand filtration system, average HPC numbers detected in the unfiltered rainwater samples ranged from 4.2×10^3 CFU/ mL on day one to 4.5×10^2 CFU/ mL on day twelve. Heterotrophic bacteria detected in filtered rainwater samples ranged from 1.2×10^4 CFU/ mL detected on day one to 5.5×10^2 CFU/ mL on day twelve of slow sand filtration. All HPC numbers increased after filtration with the exception of one filtered rainwater sample collected on day ten which decreased by 50%.

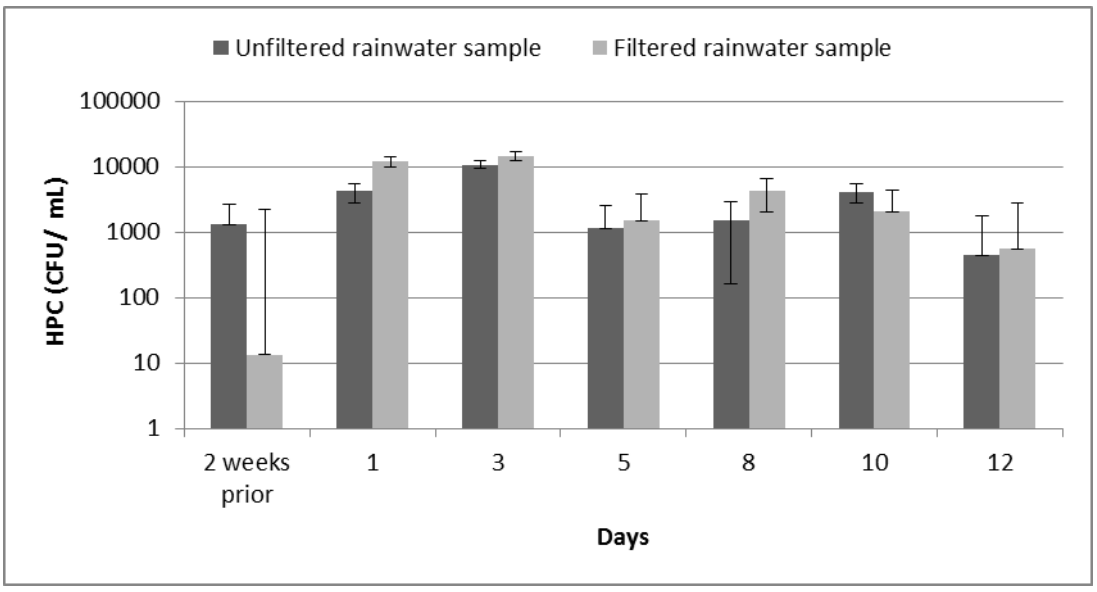


Figure 5.11. The HPC numbers enumerated before the growth of a biofilm (two weeks prior) on sand used in a slow filtration system followed by six days of monitoring the efficacy of the slow sand filtration system.

No significant correlation ($R = -0.77$, $p > 0.05$) was established between the average HPC recorded for the filtered rainwater samples and an increase in time (days one to twelve), as the results for heterotrophic bacteria in unfiltered rainwater fluctuated over time. Although reductions in HPCs were observed for filtered rainwater samples collected on day ten, the reduction was not significant ($p = 0.54$).

According to guidelines stipulated by SANS 241 (2005), the Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011) and the Department of Water Affairs and Forestry (DWAF, 1996), no *E. coli* should be present in a water sample. No guideline values for total coliforms and HPC have been stipulated by the SANS 241 (2005) and the ADWG (NHMRC and NRMMC, 2011), however, DWAF (1996) have stipulated that total coliform and HPC should not exceed 5 CFU/100 mL and 100 CFU/ mL, respectively.

While, the hydraulic retention time for the slow sand filtration system was calculated as 6.25 hours, *Escherichia coli* values for all unfiltered and filtered rainwater samples using the slow sand filtration systems did not adhere to the guidelines stipulated by SANS 241 (2005), the ADWG (NHMRC and NRMMC, 2011) and DWAF (1996), with the exception that on day eight of the slow sand filtration system, untreated rainwater samples exhibited average *E. coli* numbers of 2 CFU/100 mL which decreased to no *E. coli* detected after slow sand filtration (which was within the respective drinking water standards). Moreover, HPC and total coliform values for all unfiltered and filtered rainwater samples using the slow sand filtration systems did not adhere to the guidelines stipulated by DWAF (1996). However, total coliform values were reduced to within the

DWAF (1996) guidelines by the slow sand filtration system before the biofilm had been allowed to establish for two weeks.

5.3.3.3 Activated Carbon and Nanofibre Membrane System

A column containing two PVA membrane layers surrounded by activated carbon was connected to the rainwater tanks B and C, respectively. Total coliforms, *E. coli* and HPC numbers were detected in unfiltered and five litres of filtered rainwater samples, collected litre by litre (Table 5.9).

Total coliform counts in the unfiltered rainwater samples collected from rainwater tanks B and C had an average of 6×10^2 CFU/100 mL. After filtration, total coliform numbers were reduced significantly ($p = 0.008$) to no total coliforms detected in the first litre of filtered rainwater, less than 1 CFU/100 mL detected for the second and third litres of filtered rainwater, 1 CFU/100 mL for the fourth litre of filtered rainwater and a slight increase to 3 CFU/100 mL for the fifth litre of filtered rainwater. A 100% decrease was thus observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. From the second until the fifth litre of filtered rainwater a 99.9% to a 99.5% decrease was observed in comparison to the unfiltered rainwater samples.

Table 5.9. Total coliforms, *E. coli* and HPC numbers detected in unfiltered rainwater (n = 4) and five litres of rainwater filtered through a column containing two PVA membrane layers surrounded by activated carbon rainwater samples (n = 20).

Indicator bacteria	Unfiltered rainwater	Filtered litres of rainwater				
		1 L	2 L	3 L	4 L	5 L
Total coliforms (CFU/100 mL)	6×10^2	0	<1	<1	1	3
E. coli (CFU/100 mL)	3	0	0	0	0	0
HPC (CFU/mL)	3×10^4	3.3	1×10^2	7.8×10^1	1.5×10^2	1.6×10^2

An average of 3 CFU/100 mL *E. coli* counts were detected in the unfiltered rainwater samples collected from rainwater tanks B and C. After filtration, *E. coli* numbers were reduced significantly ($p < 0.000006$) to no *E. coli* detected in the subsequent five litres of filtered rainwater. A 100% decrease for all the five litres of filtered rainwater samples was observed in comparison to the unfiltered rainwater sample.

An average of 3×10^4 CFU/ mL heterotrophic bacterial counts was detected in the unfiltered rainwater samples collected from rainwater tanks B and C. After filtration, heterotrophic bacterial numbers were reduced significantly ($p = 0.008$) to an average of 3 CFU/ mL HPC for the first litre of filtered rainwater, 1×10^2 CFU/ mL for the second litre of filtered rainwater, 7.8×10^1 CFU/ mL for the third litre of filtered rainwater, 1.5×10^2 CFU/ mL for the fourth litre of filtered rainwater

and 1.6×10^2 CFU/ mL for the fifth litre of filtered rainwater. A 99.99% decrease was thus observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. From the second till the fifth litre of filtered rainwater a 99.6% to a 99.5% decrease was observed in comparison to the unfiltered rainwater samples.

A column containing two PVA membrane layers, without activated carbon, was also connected to rainwater tanks B and C, respectively. Total coliforms, *E. coli* and HPC numbers were detected in unfiltered and five litres of filtered rainwater samples, collected litre by litre (Table 5.10).

Total coliform numbers with an average of 3.7×10^2 CFU/100 mL were detected in the unfiltered rainwater samples collected from rainwater tanks B and C. After filtration, total coliform numbers were reduced significantly ($p = 0.006$) to an average of 1 CFU/100 mL total coliforms for the first litre of filtered rainwater, 6.3×10^1 CFU/100 mL detected for the second litre of filtered rainwater and 7.8×10^1 CFU/100 mL for the third till the fifth litre of filtered rainwater. A 99.7% decrease was observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. From the second till the fifth litre of filtered rainwater a 82.9% to a 78.9% decrease was observed in comparison to the unfiltered rainwater samples.

Table 5.10. Total coliforms, *E. coli* and HPC numbers detected in unfiltered rainwater (n = 4) and five litres of rainwater (n = 20) filtered through a column containing a two PVA membrane layers (without activated carbon).

Indicator bacteria	Unfiltered rainwater	Filtered rainwater samples				
		1 L	2 L	3 L	4 L	5 L
Total coliforms (CFU/100 mL)	3.7×10^2	1	6.3×10^1	7.8×10^1	7.8×10^1	7.8×10^1
E. coli (CFU/100 mL)	2.2	0	<1	<1	<1	1.1
HPC (CFU/ mL)	1.6×10^4	1.1×10^2	3×10^3	4×10^3	1.3×10^3	3.1×10^3

The number of *E. coli* detected in the unfiltered rainwater decreased to no *E. coli* detected in the first litre of rainwater filtered and then less than 1 CFU/100 mL for the second, third and fourth litres of filtered rainwater. The final litre of filtered rainwater contained 1.1 CFU/100 mL *E. coli*. A 100% decrease was thus observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. From the second until the fifth litre of filtered rainwater a 81.1% to a 50.8% decrease was observed in comparison to the unfiltered rainwater samples.

No significant decrease ($p = 0.15$) was however, observed in *E. coli* numbers for filtered rainwater samples with only the first litre of filtered rainwater results within the guidelines stipulated by SANS 241 (2005), the ADWG (NHMRC and NRMMC, 2011) and DWAF (1996) standards.

Heterotrophic bacterial numbers with an average of 1.6×10^4 CFU/ mL were detected in the unfiltered rainwater samples collected from rainwater tanks B and C. After filtration, heterotrophic bacterial numbers were reduced to an average of 1.1×10^2 CFU/ mL HPC for the first litre of filtered rainwater, 3×10^3 CFU/ mL for the second litre of filtered rainwater, 4×10^3 CFU/ mL for the third litre of filtered rainwater, 1.3×10^3 CFU/ mL for the fourth litre of filtered rainwater and 3.1×10^3 CFU/ mL for the fifth litre of filtered rainwater. A 99.3% decrease was observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. However, from the second till the fifth litre of filtered rainwater a 81.6% to a 81% decrease was observed in comparison to the unfiltered rainwater samples.

Based on the results obtained, the addition of activated carbon to the PVA nanofibre/activated carbon column rendered the system more efficient in the removal of total coliforms, *E. coli* and heterotrophic bacteria compared to the PVA nanofibre column without the activated carbon. For example in the first litre of filtered water the PVA nanofibre/activated carbon column removed 99.99% of the heterotrophic bacteria while the PVA nanofibre column removed 99.5%. In the fifth litre of filtered water the efficiency of PVA nanofibre/activated carbon column was 99.5% while the PVA nanofibre column efficiency was only 81%. It is therefore recommended that the addition of activated carbon serves as a pre-filter for larger particles as it increases the efficiency of the system.

Microfiltration with the use of electrospun nanfibres onto a substrate has been used in many water filtration applications. As noted previously by Bjorge et al. (2010) the increase in porosity and the pore structures formed, offer higher water permeability compared to conventional methods currently used. In this same study, general hospital wastewater, water from a pond and collected rainwater were all filtered. The microfiltration system was designed to include a hydraulic resistance time of 0.5 d. When the efficiency of the nanofibre membrane in removing culturable organisms and coliforms was compared to other microfiltration membranes, a reduction 1.5 $\log_{10}$ was observed. For the other membranes a 2 $\log_{10}$ to a 4 $\log_{10}$ removal was observed (Gómez et al., 2006; Zodrow et al., 2009). Daels et al. (2011) also noted that after filtering hospital wastewater a 2 to 3×10^1 CFU/100 mL reduction using a non-functionalised membrane was observed.

5.3.4 The Detection of Coliphages in Harvested Rainwater

The number of coliphages present in the harvested rainwater samples measured throughout this study were below the detection limit. However, when rainwater samples (500 mL) were spiked with 10 mL of *E. coli* ATCC 13706 (that had been incubated at 37˚C until log phase reached) 1 PFU/ mL was detected. These results were thus not significant and overall coliphages could not be detected in any of the rainwater samples before and after filtration. The same observations were

made by a previous study aimed at analysing, amongst others, one rainwater source in South Africa, whereby no coliphages were isolated from any of the rainwater samples. The strain of *E. coli* used in the current study, strain WG4, was also reported to be more effective in yielding coliphages counts than *E. coli* strain K12 (Nevondo and Cloete, 1999).

5.3.5 Bacterial Genus Specific PCR Reactions

5.3.5.1 Biological Filtration: Slow Sand Filtration

Two slow sand filtrations systems were connected to rainwater tanks B and C. After biofilm formation, the before and after rainwater filtered samples collected on days one, three, five, eight, ten and twelve, were analysed using genus specific PCR. The bacterial genera detected in the unfiltered and filtered rainwater samples are indicated in Table 5.11. No *Shigella* spp. or *Salmonella* spp. were detected in the unfiltered or the filtered rainwater samples collected throughout the study period.

In summary *Aeromonas* spp. (GenBank accession no. EF450824.1, CP005966.1) were the least dominant species and were detected in 17% of the unfiltered rainwater samples, with no *Aeromonas* spp. detected in the slow sand filtration samples. Slow sand filtration was however, not effective in removing *Yersinia* spp. as *Yersinia* spp. (GenBank accession no. HM142628.1) were detected in 58% of the filtered rainwater samples. *Pseudomonas* (GenBank accession no. JX279939.1) were one of the more dominant genera detected in this study with the use of genus specific PCRs and were detected in 92% of the unfiltered rainwater samples. Slow sand filtration was however, ineffective in removing *Pseudomonas* spp. as PCR assays confirmed the sporadic presence of *Pseudomonas* spp. in 75% of the filtered rainwater samples. Similarly, *Klebsiella* (GenBank accession no. EU430287.1) were also one of the more dominant genera detected in this study with the use of genus specific PCR as *Klebsiella* were detected in all the unfiltered rainwater samples and slow sand filtration was again ineffective in removing *Klebsiella* spp. from rainwater as this genera was sporadically detected in 92% of the filtered rainwater samples. *Legionella* (GenBank accession no. AB638719.1) were the most dominant genera detected throughout this study and slow sand filtration was ineffective in removing *Legionella* spp. as *Legionella* spp. were detected in all the filtered and unfiltered rainwater samples collected.

Table 5.11. Bacteria genera detected in unfiltered and slow sand filtered rainwater samples with the use of PCR

Organism	Unfiltered Rainwater Sample (%)	Filtered Rainwater Sample (%)
Aeromonas spp.	17	0
Klebsiella spp.	100	92
Legionella spp.	100	100
Pseudomonas spp.	92	75
Salmonella spp.	0	0
Shigella spp.	0	0
Yersinia spp.	42	58

With the exception of *Aeromonas* spp., genus specific PCR assays revealed that the slow sand filtration system was ineffective in removing pathogenic bacteria commonly associated with rainwater. The biofilm known as the schmutzdecke, is a biologically active layer that forms at the sand water interface (Campos et al., 2002). Joubert and Pillay (2008) suggested that a ripening period of six to eight weeks was required for the schmutzdecke to mature and in shortened time periods the schmutzdecke has been shown to be sub-optimal. As the slow sand filter utilised in the current study was ineffective in removing bacterial indicators it is hypothesised that the biofilm layer had not reached full maturation. Slow sand filters have however, been effective in reducing for example, nitrates (Aslan and Cakici, 2007), antimicrobial contaminants (Rooklidge et al., 2005) as well as pathogenic bacteria such as *E. coli*, *Vibrio cholerae* and *Salmonella typhimurium* (Mwabi et al., 2011). Although many faster and more effective filtration methods exist, slow sand filtration is generally considered cost effective, easy to operate, requires minimal maintenance and has shown to be effective in removing pathogenic bacteria in other studies (Joubert and Pillay, 2008). For these reasons, slow sand filtration is an attractive alternative point of use treatment system in developing countries and rural communities (Logsdon et al., 2002).

5.3.5.2 Activated Carbon and Nanofibre Membrane System

A column containing two PVA membrane layers surrounded by activated carbon were connected to rainwater tanks B and C, respectively. Total DNA was extracted from unfiltered and five litres of filtered rainwater samples, collected litre by litre, followed by subsequent genus specific PCR analysis.

Genus specific PCR assay revealed the presence of certain potentially pathogenic bacteria, commonly associated with rainwater (Table 5.12). Throughout this study, no *Salmonella* spp. were detected in any of the filtered and unfiltered rainwater samples, and *Shigella* spp. (GenBank accession no. HE616529.1) were detected in 25% of the third litre of filtered rainwater samples.

Of the reoccurring genera, *Aeromonas* spp. (GenBank accession no. CP005966.1) were detected in all the rainwater samples collected before filtration, and were reduced to no *Aeromonas* spp. detected in the first litre of filtered rainwater, and thereafter were detected in 25% of the remaining filtered rainwater samples, from the second to the fifth litre of filtered rainwater.

Table 5.12. Bacteria genera detected in unfiltered and filtered rainwater samples of filtration through the activated carbon and nanofibre membrane system.

Organism	**Unfiltered rainwater (%)**	**Filtered liters of rainwater (%)**				
		1st	**2nd**	**3rd**	**4th**	**5th**
Aeromonas spp.	100	0	25	25	25	25
Klebsiella spp.	100	25	75	50	50	75
Legionella spp.	100	75	100	75	75	100
Pseudomonas spp.	100	50	50	75	25	25
Salmonella spp.	0	0	0	0	0	0
Shigella spp.	0	0	0	25	0	0
Yersinia spp.	100	75	50	100	100	75

In summary, *Klebsiella* spp. (GenBank accession no. X16817.1) were detected in all the unfiltered rainwater samples and after filtration were detected in 25% after the first litre of rainwater had been filtered and in 75% of the second and fifth litre of filtered rainwater, respectively. During the filtration of the third and fourth litres of rainwater, *Klebsiella* spp. were detected in 50% of the rainwater samples filtered, respectively. Similar to the detection of *Klebsiella* spp., *Pseudomonas* spp. (GenBank accession no. HF952526.1) were detected in all the unfiltered rainwater samples and after filtration were detected in 50% of the first and second litres of filtered rainwater samples and in 75% of the third litre of filtered rainwater sample. *Pseudomonas* spp. were also detected in 25% of the fourth and fifth litres of rainwater samples filtered.

Of the dominant bacteria detected, *Yersinia* spp. (GenBank accession no. HM142628.1) were detected in all the unfiltered rainwater samples as well as all the samples collected for the third and fourth litres of filtered rainwater. *Yersinia* spp. were also detected in 50% (second litre) and in 75% (first and fifth litres) of the respective filtered rainwater samples. *Legionella* spp. (GenBank accession no. JN381009.1, HQ111823.1, HQ711922.1, HQ112142.1) were also dominant and PCR assays confirmed the presence of *Legionella* in all the unfiltered rainwater samples as well as all the second and fifth litres of filtered rainwater. *Legionella* spp. were also detected in 75% of the first, third and fourth rainwater litres that were filtered.

Microfiltration is currently being widely applied in water treatment. Due to size, *Staphylococcus aureus* (0.8µm x 1 µm) and *E. coli* (2 µm x 1 µm) are presumed not likely to pass through the nanofibre membrane with a mean pore size of 0.20 – 0.45 µm. Daels et al. (2011) however noted that in hospital wastewater that had been spiked with *S. aureus*, a 1.6×10^1 CFU/100 mL reduction

was observed using a non-functionalised membrane. The average pore size of the membrane used in this study was larger (0.9) µm than 0.2 – 0.45 µm (Daels et al., 2011), and for this reason two layers of the nanofibre membrane was used in this filtration system. It is therefore possible that the pore size did not decrease after doubling the layers of the membrane and even with the addition of activated carbon, the pore size could possibly not have been reduced to 0.2 – 0.45 µm. It should however be noted as indicated by the recovery of indicator bacteria that the first litre of filtered rainwater was within DWAF (1996) standards.

5.4 Conclusions:

The aim of this study was to access the efficiency of four water treatment systems in the removal of rainwater contaminants. Activated carbon based disinfection systems, included a slow activated carbon filtration system and an activated carbon/PVA nanofibre column. Other disinfection systems included a slow sand filtration system and a PVA nanofibre (without activated carbon) column. All systems were analysed for harvested rainwater treatment. The microbial parameters that were investigated for all disinfection systems included the enumeration of total coliforms, *Escherichia coli* and heterotrophic bacteria. In addition, the treated and untreated rainwater samples collected for the slow sand filtration and activated carbon/PVA nanofibre column were screened for the presence of selected pathogenic bacteria using molecular techniques. The chemical parameters that were investigated during the pilot scale study included the concentration of metal ions, anions and cations present in the treated and untreated harvested rainwater samples.

Chemical analysis of the activated carbon based systems indicated that all cations and anions present in both the unfiltered and filtered rainwater samples were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of aluminium and antimony detected in rainwater samples filtered through the slow activated carbon system. Moreover, all cations and anions present in the rainwater samples collected before and after slow sand filtration were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of aluminium, manganese and iron. These four cations were shown to increase significantly ($p < 0.05$) in the filtrate of the biological filtration systems and it is hypothesised that these elements could have leached from the commercially available filtration media of each system. As increased concentrations of aluminium, antimony, magnesium and iron were only detected on the first day of sampling and not on the last day, it is recommended that rainwater should be allowed to filter through the system for at least two weeks before use for domestic and irrigation purposes.

While a decrease in heterotrophic bacteria and total coliforms was observed due to the slow activated carbon filtration system, the decrease was not significant. *Escherichia coli* numbers, however, were shown to have decreased significantly ($p < 0.05$) when utilising these filtration systems, but the reduction was not to within drinking water guidelines. In addition heterotrophic bacteria, total coliforms and *E. coli* still persisted in filtered rainwater samples collected after five days of maintaining the filtration systems.

All heterotrophic bacteria, total coliforms and *E. coli* persisted in filtered rainwater samples collected after twelve days of maintaining the slow sand filtration system. Based on genus specific PCR analysis, utilised to screen filtered rainwater samples for the presence of pathogenic bacteria that have previously been detected in harvested rainwater (Chapter 2), no *Salmonella* spp. or *Shigella* spp. were detected in any of the rainwater samples analysed. *Aeromonas* spp. were the least dominant species detected and were only detected in unfiltered rainwater samples. Of the more dominant genera detected, *Yersinia* spp., *Klebsiella* spp. and *Pseudomonas* spp. were identified in more than half of the filtered rainwater samples analysed, including the last filtered sample collected after twelve days. Moreover the slow sand filtration system had no effect on the removal of *Legionella* spp. as PCR assays confirmed the presence of *Legionella* in all unfiltered and filtered rainwater samples.

It is therefore recommended that the biological filtration systems utilising activated carbon and silica sand, be analysed further for a longer time period to monitor the effectiveness of a mature biofilm in the removal of pathogenic bacteria. The biological filtration systems have practical limitations such as the extended time required for the maturation of the biofilm, sporadic sloughing of the biofilm during filtration and the need for a continuous flow of rainwater. However, these systems are cost-effective and can be easily maintained.

While the chemical quality of the rainwater was generally lower than the stipulated drinking water guidelines, results for the microbial quality of rainwater filtered through the activated carbon/PVA nanofibre column indicated that while a four log reduction was obtained in the first filtered litre, the heterotrophic bacteria persisted in the four subsequent filtered litres of rainwater. However, total coliforms were reduced to zero (100%) for the first litre of filtered rainwater and *E. coli* were reduced to zero (100%) in each of the five filtered rainwater samples. Based on genus specific PCR analysis, utilised to screen the five litres of filtered rainwater samples for the presence of pathogenic bacteria, no *Salmonella* spp. were detected in any of the rainwater samples analysed. *Shigella* spp. were only detected in the third litre of filtered rainwater. *Aeromonas* spp. were removed in the first litre of filtered rainwater and were detected in all the unfiltered rainwater samples. However,

Klebsiella spp. *Legionella* spp., *Pseudomonas* spp. and *Yersinia* spp. were not removed by the activated carbon/PVA nanofibre column as these organisms where detected in all five litres of the filtered rainwater and all the unfiltered rainwater samples. Assays will however have to be conducted in order to confirm whether the organisms were viable. In addition, the PVA nanofibre membrane system with the addition of activated carbon needs to be further optimised, as only one litre of potable water can be produced before the components of the system need to be replaced. Moreover, the average time required to filter one litre of rainwater is approximately 37.48 minutes, rendering this system impractical for use at a household level.

The addition of activated carbon to the first PVA system may have provided an additional filtration barrier, as based on the microbial analysis performed to monitor the second PVA nanofibre (without activated carbon) column, all five litres filtered could not be used for potable purposes. Heterotrophic bacteria and total coliforms persisted in all five litres of filtered rainwater, and while *E. coli* numbers were reduced (100%) in the first litre of filtered rainwater, *E. coli* were not removed completely in the remaining four litres of filtered rainwater. The number of coliphages present in the unfiltered and filtered harvested rainwater samples, were below the detection limit throughout the study period.

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Chapter 6:

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(Chapter 6 is thus compiled in the format of the Water Research Commission report and UK spelling is employed)

Efficiency of a Solar Pasteurization System in Disinfecting Harvested Rainwater

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Abstract

Many studies have concluded that harvested rainwater is susceptible to chemical and microbial contamination. The first phase of the study was thus aimed at pasteurizing rainwater samples in laboratory scale experiments. Analysis of results showed that the thermal death time of the heterotrophic bacteria in harvested rainwater was 30 minutes at a treatment temperature of 72˚C. In addition, the majority of the phycrophiles and thermophiles isolated from heat treated rainwater samples belonged to the *Bacillaceae* family. The aim of the second phase of the study was then to monitor the efficiency of a solar pasteurization system in reducing the microbiological load in harvested rainwater and to determine the change in chemical contaminants after rainwater had undergone pasteurization. Three rainwater harvesting tanks were installed on the Welgevallen Experimental farm, Stellenbosch, South Africa. A solar pasteurization system was connected to one of the rainwater harvesting tanks and unpasteurized as well as pasteurized rainwater samples were collected for chemical and microbial analysis. The temperature ranges of the pasteurized rainwater samples were 55 to 57˚C, 64 to 66˚C, 72 to 74˚C, 78 to 81˚C and 90 to 91˚C. All cations were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of iron (55˚C, 65˚C, 78˚C, 91˚C) aluminium (78˚C), lead (55˚C, 65˚C, 91˚C) and nickel (55˚C, 65˚C, 78˚C) which were detected in the pasteurized rainwater samples and were above the respective guidelines. Indicator bacteria including, heterotrophic bacteria, *Escherichia coli* and total coliforms where reduced to zero at pasteurization temperatures of 72˚C and above. However, with the use of molecular techniques *Yersinia* spp., *Legionella* spp., and *Pseudomonas* spp., were detected in rainwater samples pasteurized at temperatures greater than 72˚C. It is therefore recommended that the storage tank of the pasteurization system be made of an alternative material that will not impact the rainwater negatively.

Key Words: Rainwater harvesting; pasteurization; pathogens; chemical and microbial quality

6.1 Introduction

Water resources are becoming increasingly scarce throughout the world due to population increases, climate change and contamination caused by point- and non-point source pollution (Alam Imteaz et al., 2011). While access to safe water and sanitation services were identified as part of the Millennium Development Goals (MGD), currently it is estimated that approximately 9% of the population in South Africa do not have access to a sufficient water supply infrastructure, while approximately 22 million (43%) people have insufficient sanitation services (Census, 2011). In addition, the South African government struggles to meet the water requirements of the large number of inhabitants in rural and many peri-urban and urban areas. Alternative and sustainable sources of water must thus be considered to supply freshwater for domestic and potable purposes (Le vesque et al., 2008).

Alternative water resources include storm water harvesting, grey water and wastewater reuse as well as desalination. Storm water harvesting is utilised most often and includes rainwater harvesting and river water utilisation (Alam Imteaz et al., 2011). While these alternate water sources are routinely utilised in many countries all over the world (Nevondo and Cloete, 1991), in South Africa water sources such as rainwater and river water are severely contaminated and it is therefore not advisable to use this water as a primary potable water source (Mwenge Kahinda et al., 2007). In addition, untreated water could cause diseases, which poses a threat to new-borns, young children, the elderly, immuno-compromised people and people living in unsanitary conditions (Mwenge Kahinda et al., 2007). It is also estimated that approximately 38 000 South Africans die each year from diarrhoeal diseases associated with contaminated water (Sciencescope, 2009). Domestically harvested rainwater may thus provide an alternative source of drinking water, but only if the water meets the international standards of drinking water as stipulated by the World Health Organisation (WHO, 2008). In order to provide clean and safe drinking water to rural communities and informal settlements in urban areas, the development of effective water treatment methods are required.

Solar pasteurization can be utilised as a possible treatment for contaminated water sources as microorganisms are susceptible to heat (pasteurization) and ultraviolet-A radiation. The sun is a free, natural source of energy and its full potential remains untapped. Solar pasteurization (SOPAS) differs from solar disinfection (SODIS) in that the SOPAS reactor inactivates microorganisms by only using the thermal effect at a temperature of at least 70°C without radiation, whereas the SODIS reactor uses both the thermal effect and UV-A radiation (Sommer et al., 1997).

According to Nieuwoudt and Mathews (2005) the technology of heating water to below boiling temperature has gained much interest and for this reason the design and implementation of the heat based disinfection systems is fairly advanced. Currently, there are three types of water heating systems that are manufactured predominantly for domestic use (Solar Energy Equipment, 2000; SANS 1307, 2003). The most expensive system is a split system of two components, a collector and a storage tank, where water is heated directly or indirectly. The collector is usually installed on the north-facing area of the roof and the storage tank inside the roof. This allows for a thermo-siphon effect whereby water is able to circulate through the collector due to the ranges of temperatures. This is a passive system and for this reason an electric pump for circulating water is not required. The second closed coupled system is comprised of a flat plate collector, that can heat water directly or indirectly, and a separate elevated storage tank attached to the end of the collector. It has been noted that these systems are less expensive and installation is easier than the split systems. Lastly, a less efficient system closed system, is the integrated collector storage, or the integral collector (ICS) system comprising of a collector that is used to heat and store the water. These systems are the most cost effective. Close-coupled systems are also usually placed on the north-facing section of pitched roofs. For this system, both the flat plate collector and the storage tanks are exposed. Being a passive system, again the water moves via the thermo-siphon effect (Nieuwoudt and Mathews, 2005).

The aims of this study were to assess the efficiency of different pasteurization temperatures (laboratory scale treatment of rainwater samples) and a heating based disinfection system, namely a closed coupled system [pilot scale treatment of rainwater samples directly from a domestic rainwater harvesting (DRWH) tank] in treating harvested rainwater. The microbial parameters that were investigated during the laboratory scale experiments included the enumeration of heterotrophic bacteria as well as the identification of microbial isolates able to withstand heat treatment. During the second phase of the study microbial parameters that were investigated included the enumeration of total coliforms, *Escherichia coli* and heterotrophic bacteria. In addition, the treated and untreated rainwater samples of the pilot scale study were screened for the presence of selected pathogenic bacteria using molecular techniques. The chemical parameters that were investigated during the pilot scale study included the concentration of metal ions, anions and cations present in the treated and untreated harvested rainwater samples.

6.2 Materials and Methods

6.2.1 Phase 1: Preliminary Pasteurization Study

A preliminary pasteurization study was conducted in the Environmental Microbiology laboratory of the Department of Microbiology at Stellenbosch University.

6.2.1.1 Sample Site and Collection

Rainwater samples were collected from the rainwater harvesting tank (2000 L) installed outside the JC Smuts building of Stellenbosch University. Samples were then heated at various temperature and time intervals in order to determine the optimum range at which the heterotrophic plate counts (HPC), which serves as an indicator for disinfection, were reduced to within drinking water standards as stipulated by South African Water Quality Guidelines for Domestic Water Use of the Department of Water Affairs and Forestry (DWAF, 1996) and the Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011). Re-growth of the heterotrophic bacteria was also monitored by incubating the respective plates at different temperatures for varying time periods. In addition, the bacterial groups that were able to withstand extreme temperature conditions were isolated and identified.

6.2.1.2 Laboratory-scale Pasteurization Experiments and Recovery of Heterotrophic Bacteria

In order to analyse the efficiency of thermal heat inactivation and the corresponding time interval in reducing bacterial numbers, rainwater samples were pasteurized as follows: Conical flasks containing 100 mL rainwater samples collected from the DRWH tank installed outside the JC Smuts building, Stellenbosch University, were incubated in a water bath at 50˚C, 55˚C, 60˚C, 65˚C and 72˚C, respectively for five minute intervals (after 30 min). It is important to note that: the rainwater samples were placed in the water baths and were heated to the respective temperatures, once the rainwater sample had reached the same temperature as the water bath, this was designated as time zero (t = 0). An undiluted and a 10-fold dilution of the untreated and treated rainwater were then prepared for each temperature at the different time intervals and spread plated (100 µL) onto Nutrient Agar (NA) and R2A agar (Difco). To ensure that there was no cross contamination of samples and to eliminate false positive results, a control sample of sterile water that had been autoclaved at 121˚C, 100 kPa (15 psi) for 20 minutes was also included and plated out each time a treated rainwater sample was taken. The various incubation temperatures and incubation periods are summarised in Table 6.1. According to La Duc et al. (2007) for the recovery of psychrophiles, mesophiles and thermophiles, incubation should be conducted at the following temperatures ≤15˚C, 20 - 45˚C, and 55 - 65˚C, respectively. Moreover, in order to recover slow growing bacteria from

environmental samples, incubation time periods will also vary, for example for the enumeration of psychrophiles it is suggested that the incubation period be up to 10 days in order to recover all bacteria able to grow in colder temperatures.

Table 6.1. Classification of bacteria based on a physiological characteristic (cardinal temperature) (La Duc et al., 2007)

Descriptive Term	Definition (˚C)	Incubation Temperature (˚C)	Incubation Time (days)
Psychrophile	Grows at 0˚C, optimum ≤15˚C	4	10
Mesophile	Optimum 20 - 45˚C (25˚C)	37	4
Thermophile	Grows ≥55˚C, optimum 55 and 65˚C	56	4

6.2.1.3 Genomic DNA Extractions from Isolates

Isolates were selected based on differences in colour reactions and morphological characteristics and sub-cultured onto NA at least three times. Once pure colonies had been obtained, a single colony was inoculated into 5 mL Nutrient Broth (Merck) and incubated at 37°C overnight. Cells were harvested by centrifuging 2 mL of each culture for 10 min at 6000 × g. The extraction of genomic DNA from each isolate was performed using the ZR^{TM} Soil microbe DNA Miniprep Kit (Zymo Research) according to the manufacturer's instructions. The genomic DNA was then visualised on a 0.8% agarose gel stained with 0.5 µg/mL ethidium bromide. Electrophoresis was conducted at 80 volts for approximately one hour with the use of 1X Tris/Borate/EDTA (TBE) buffer (Sambrook et al., 1989).

6.2.1.4 Polymerase Chain Reactions Used to Identify Isolates

Polymerase Chain Reactions (PCR) were used to amplify the 16S rRNA conserved sequence of each isolate (Rawlings, 1995). The PCR mixture consisted of a final volume of 50 µL and contained 10 µL of 5X Green GoTaq® Flexi Buffer (1X) (Promega), 4 µL $MgCl_2$ (2.0 mM) (Promega), 0.5 µL of each dNTP (0.1 mM) (Thermo Scientific), 0.5 µL of the PCR primers (0.1 µM) mentioned in Table 6.2, 0.3 µL of GoTaq® Flexi DNA Polymerase (Promega) (1.5U), and 2 µL of genomic DNA per respective isolate. A DNA Thermal Cycler (Bio-Rad, USA) was used throughout this study to perform PCR reactions. Amplification was performed using an initial template denaturation step at 94°C for 3 min and then 30 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s and extension at 72°C for 1.5 min, with a final extension for 5 min at 72°C (Rawlings, 1995).

Table 6.2. Sequences of universal primer sets utilised (Rawlings, 1995)

Organism	Primer Name	Primer Sequence (5'-3')	Gene (Size)
Universal	fDD2 rPP2	CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG CCAAGCTTCTAGACGGITACCTTGTTACGACTT	16S rRNA (1.6 Kb)

PCR products were analysed by gel electrophoresis in 0.8% agarose (Bio- Rad) containing 0.5 µg/mL ethidium bromide in 1X TBE buffer. DNA bands were confirmed by UV illumination and photographed using the Gel Doc 1000 documentation system (Bio-Rad). Once the size and the concentration of the PCR products had been confirmed, the products were cleaned and concentrated using the DNA Clean & Concentrator™-5 Kit (Zymo Research) as per manufacturer's instructions. The cleaned products were then sent to the Central Analytical Facility (CAF) at Stellenbosch University for sequencing. Chromatograms of each sequence were examined using FinchTV v. 1.4.0 software and were aligned using DNAman™ version 4.1.2.1 software. Sequence identification was completed using the National Centre for Biotechnology Information (NCBI) and The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest match of local similarity between isolates and the international database in GenBank, EMBL, DDBJ and PDB sequence data (Altschul et al., 1990). The sequences of representative isolates, that showed > 97% similarity (< 3% diversity) to organisms was recorded.

6.2.2 Phase 2: Pilot Scale Pasteurization System

Based on the results obtained in the laboratory-scale experiments (Section 6.2.1), a solar pasteurization system (Apollo Solar Technology Pty. Ltd., South Africa) was attached directly to the DRWH tank A (Figures 6.1 and 6.2) set up on the Welgevallen Experimental farm, Stellenbosch University.

6.2.2.1 Sample Site and Collection

Three polyethylene domestic rainwater harvesting (DRWH) tanks (2000 L) were installed at the Welgevallen Experimental farm, Stellenbosch University, South Africa (Figure 6.1). The farm is situated on the periphery of the town of Stellenbosch. As indicated in Figure 6.1, the sampling site was surrounded by trees, however no tree branches obstructed the catchment area. The farm was also surrounded by dirt roads that were continuously used by motor vehicles and the farm workers to herd cattle twice a day, as the tanks were situated on the northern side of a well-established building that neighboured the farms' dairy. In addition, due to damage, a section of the guttering lengths on the building where the DRWH tanks were installed had to be replaced, and new down pipes were installed in order to link the tanks to the old gutter system surrounding the catchment

area. The older gutter system was constructed from white asbestos (Chrysotile) and the roofing was constructed from corrugated galvanized iron.

For ease of sampling tank A was installed on a metal stand so that the rainwater could flow from the tank into the solar pasteurization system in a passive manner. This pasteurization system was donated to Stellenbosch University by a company in Somerset West, Crest. The middle tank (tank B) and the right end tank (tank C) were used to test the filtering systems (discussed in Chapter 5).

Figure 6.1. The sampling site (33°56'36.19"S, 18°52'6.08"E) used in Phase 2 of the study was located at Welgevallen Experimental farm, Stellenbosch, South Africa. Three domestic rainwater harvesting tanks were installed: (A) Solar pasteurization system and (B) and (C) where various treatment systems were intermittently connected (Chapter 5).

For microbial and chemical analysis before and after solar pasteurization, rainwater samples were collected in 1 L sterile Schott bottles. The temperature and pH of the rainwater at the sampling locations were measured using a hand-held mercury thermometer and colour-fixed indicator sticks with a pH range of 0 - 14 (ALBET®, Barcelona, Spain). A MadgeTech TC101A - thermocouple temperature Data Logger (Madge Tech, Inc) was installed to monitor the temperature of the rainwater inside the storage tank of the solar pasteurization system. To ensure that only the less dense warm water was being monitored, the probe of the logger was passed through the inlet tank and approximately half way into the storage tank (indicated by a black arrow in Figure 6.2). The temperature data obtained from the log tagger was analysed using Data Logger Software version 4.1.5. Rainfall and temperature patterns were obtained from the South African Weather Services

(SAWS, 2013), while direct solar radiation data was obtained from Stellenbosch Weather Services, Engineering Faculty (http://weather.sun.ac.za/).

Samples were collected from July 2013 until October 2013 (Table 6.3) at various temperature ranges (55 to 57˚C; 64 to 66˚C; 72 to 74˚C; 78 to 81˚C; 90 to 91˚C). For each temperature range, three sampling events were performed with a total of 15 sampling events conducted. For each temperature, untreated and 1 L of heat treated rainwater samples were collected in duplicate.

Table 6.3. Sampling dates and temperatures of the solar pasteurized samples analysed

Sampling Date	Temperature of Pasteurized Water Sample (˚C)
11.07.2013	56
22.07.2013	57
30.07.2013	65
19.08.2013	55
20.08.2013	81
26.08.2013	78
02.09.2013	64
06.09.2013	81 (2^{nd})
06.09.2013	91
10.09.2013	66
10.09.2013	74
11.09.2013	72
11.09.2013	90
12.09.2013	91 (2^{nd})
09.10.2013	73

6.2.2.2 Solar Pasteurization System

The Apollo™ solar pasteurization system was designed and manufactured in China and donated to Stellenbosch University by a company in Somerset West, Crest. The information regarding the solar pasteurization system was obtained from http://www.apollotechnology.co.za/low_pressure_system.php. The water from the rainwater tank A flows through the system components (Figure 6.2) as follows; firstly, cold water flows from the rainwater tank through the cold water feed (A) into the cold water stainless steel inlet tank (C). To increase the flow rate into the inlet tank it is suggested that larger, shorter pipes with gentle bends be used in the system. From the inlet tank, cold water flows into the stainless steel main storage tank (D) (capacity: 100 L) then down through the high borosilicate glass collector tubes (E). Through the principle of thermo-siphoning, as the cold water (blue arrow) heats it loses density, and becomes more buoyant, the heated water is then able to move up (red arrow) into the main storage tank again. In this manner, a natural circulation of cold water and hot water is started. If the process

of hot water being replaced by colder more dense water continues, the whole body of water in the main tank will heat up. Heated water is then harvested from the hot water outlet (F).

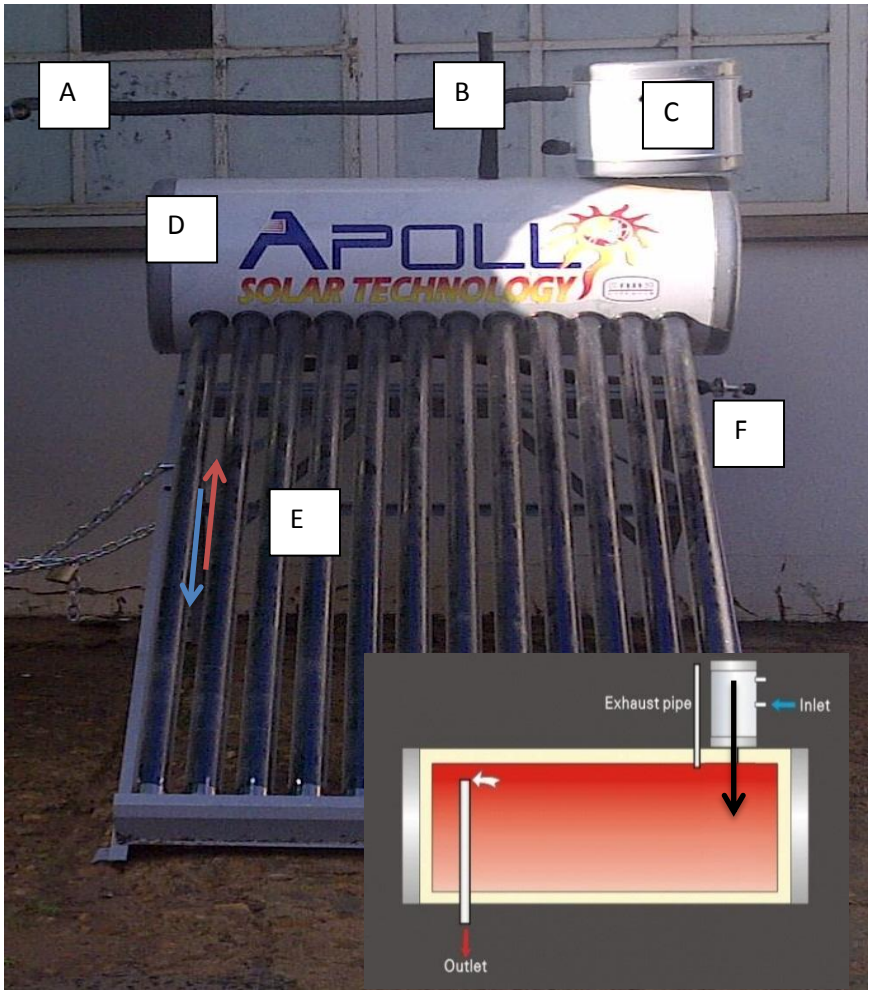

Figure 6.2. The low pressure solar pasteurization system (ASLP-12/1800-58) used in this study was donated to Stellenbosch University by a company in Somerset West, Crest. The labels and corresponding components of the solar pasteurization system included: A: Cold water feed into the tank, B: Exhaust pipe, C: Cold water inlet tank, D: Main storage tank, E: 12 × Collector tubes, F: Hot water outlet.

6.2.2.3 Chemical Analysis

Pasteurized samples collected for the various temperature ranges, namely, 55˚C, 65˚C, 78˚C and 91˚C, were analysed for the chemical parameters. For the determination of the metal concentrations, Falcon™ 50 mL high-clarity polypropylene tubes containing polyethylene caps, were pre-treated with 1% nitric acid before sampling. The concentrations of metals such as aluminium (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn), amongst others, were then determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) according to Saleh et al. (2000) and nitric acid digestion. All samples were analysed for the presence of metals at the Central Analytical Facility (CAF), Stellenbosch University.

Pasteurized rainwater samples were also sent to the Centre for Scientific and Industrial Research (CSIR) Stellenbosch for anion analyses. The anions detected along with the corresponding detection method are summarised in Table 6.4.

Table 6.4. Methods used in the detection of anions performed by the CSIR, Stellenbosch

Anion	**Method**
Nitrate and Nitrite	SALM 7.0 Automated Colorimetry
Soluble phosphate	SALM 9.0 Automated Colorimetry
Sulphate	MALS 6.5 ICP OES Detection
Chloride	SALM 1.0 Automated Colorimetry
Fluoride	SALM 11 Potentiometric measurement

6.2.2.4 Recovery of Indicator Organisms

To enumerate HPC, a serial dilution was prepared for each sample (10^{-1}–10^{-2}) and by use of the spread plate method 100 µl of each dilution was cultured onto R2A agar (Difco) in triplicate, with the plates incubated at 37°C for up to four days. Total coliforms (TC) and *Escherichia coli* (*E. coli)* were then enumerated simultaneously by filtering a total volume of 100 mL (undiluted and 10^{-1}) through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (10 kPa, 10 psi). The filters were then incubated on Membrane Lactose Glucuronide Agar (MLGA) (Oxoid, Hampshire, England) at 35 ± 2°C for 18 - 24 hours (U.S. Environmental Protection Agency, 2009). All analyses were performed in triplicate. The regrowth of bacteria for each treated sample was also monitored for one week after sampling by storing 10 mL of each sample in a test tube at room temperature and spread plating 100 µL on a daily basis onto R2A agar to enumerate HPC, which serves as a parameter of disinfection efficiency.

6.2.2.5 The Bacterial Removal Efficiency of the Pasteurization System

The bacterial removal efficiency of the system was obtained by comparing the bacterial counts obtained from the samples taken before pasteurization and the average bacterial counts obtained from samples taken after pasteurization. The log reduction was calculated using equation 1 and the percentage reduction was calculated using equation 2 (Brözel and Cloete, 1991).

Equation 1:

$$\text{Log reduction} = (\text{Log}_{10}\ \text{bacterial count}_{\ \text{before pasteurization}} - \text{Log}_{\ 10}\ \text{bacterial count}_{\ \text{after pasteurization}})$$

Equation 2:

$$\text{Percentage reduction} = 100 - \frac{\text{Survivor count}}{\text{Initial count}} \times 100$$

6.2.2.6 Recovery and Assay of Coliphages

Coliphages were enumerated according to Baker et al. (2003). Briefly, 30 µL of chloroform (BDH AnalaR) was added to 2 mL of an untreated rainwater sample as well as a solar pasteurized rainwater sample collected at the different temperatures as outlined in Table 6.3. Each sample was then centrifuged at 13200 × g for five minutes. To ensure that no chloroform was transferred, 1 mL of each sample was subsequently added to 100 µL *Escherichia coli* ATCC 13706 (Microbiologics®) which had been grown to stationary phase in Luria-Bertani Broth (Merck). After each sample had been briefly vortexed, the samples were incubated at 25˚C for five minutes. The mixture was transferred to a test tube containing 5 mL of melted top agar (7% Luria-Bertani w/v), mixed gently and poured onto a plate containing Luria-Bertani Agar. Once the plates had set, the samples were incubated at 37˚C for 18 hours. Each assay was performed in triplicate for each sample. Rainwater samples that were spiked with *E. coli* ATCC 13706 were also analysed in the same manner, for this, 10 mL of *E. coli* DH5α that had been cultured to the stationery phase was added to 500 mL rainwater, the mixture was then allowed to stand at room temperature for approximately 6 hours before samples were analysed for the presence of coliphages.

6.2.2.7 Extraction of Total DNA from Rainwater Samples

Total DNA extractions were performed for each of the 45 rainwater samples collected before and after pasteurization. In order to extract total genomic DNA from the rainwater samples a modified version of the boiling method was utilised (Watterworth et al., 2005). Each rainwater sample (500 mL) was filtered through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (10 kPa, 10 psi). The filters for each rainwater sample were then incubated in 2 mL Luria Bertani (LB) (Merck) broth for 5 hours at 37°C. The samples were vortexed for 15 min to detach the cells from the filters and cells were harvested from 2 mL of the cell suspension through centrifugation at 12500 × g for 10 min. The supernatant was discarded and the pellet re-suspended in 100 µL sterile MilliQ water. The re-suspended cells were then boiled at 95°C for 15 min, followed by cooling on ice for 10 min. The sample was subjected to centrifuging at 12500 × g for 5 min and the supernatant transferred to a sterile eppendorf tube. In each instance genomic DNA and total DNA was visualised on a 0.8% agarose gel stained with

0.5 µg/mL ethidium bromide. Electrophoresis was conducted at 80 volts for approximately one hour with the use of 1X TBE buffer (Sambrook et al., 1989).

6.2.2.8 Genus Specific PCR Reactions

Primers and PCR conditions as outlined in Table 6.5 were utilised in the current study for the identification of documented pathogenic and opportunistic bacterial pathogens. Each PCR mix was performed in a final volume of 50 µL. For the detection of *Shigella* spp., *Salmonella* spp. and *Aeromonas* spp. the PCR mix consisted of 10 µL of 5X Green GoTaq® Flexi Buffer (1X) (Promega), 4 µL $MgCl_2$ (2.0 mM) (Promega), 0.5 µL of each dNTP (0.1 mM) (Thermo Scientific), 0.5 µL of the respective PCR primers (0.1 µM), 0.3 µL of GoTaq® Flexi DNA Polymerase (Promega) (1.5U) and 10 µL of template DNA. For *Yersinia* spp. and *Klebsiella* spp. the same PCR mix was used with the exception that 1.5 µL of the respective forward and reverse PCR primers (0.3 µM) was used. For *Pseudomonas* spp. and *Legionella* spp., again, the same reaction mixture was used, however 2.0 and 2.5 µL of each PCR primer (0.4 and 0.5 µM, respectively) was used, respectively.

The following strains were cultured as positive controls after which, genomic DNA was extracted; *Legionella pneumophila* ATCC 33152, *Shigella sonnei* ATCC 25931, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Aeromonas hydrophila* (environmental strain), *Klebsiella pneumoniae* ATCC 13385 and *Yersinia enterocolitica* ATCC 27729. All positive control organisms were obtained from Microbiologics®, unless indicated otherwise. The specificity of each primer set was confirmed by using non target DNA extracted from all the above mentioned positive controls and a negative control (sterile distilled H_2O) was also included.

All PCR products were analysed by gel electrophoresis in 1.5% agarose (Bio- Rad) containing 0.5 µg/mL ethidium bromide in 1X TBE buffer. Deoxyribonucleic acid bands were confirmed by UV illumination and photographed using the Gel Doc 1000 documentation system (Bio-Rad). Once the size and the concentration of the PCR products had been confirmed, products of representatives of the samples were purified and concentrated using the DNA Clean & Concentrator™-5 Kit (Zymo Research) as per manufacturer's instructions. The cleaned products were then sent to the Central Analytical Facility at Stellenbosch University for sequencing. Chromatograms of each sequence were examined using FinchTV v. 1.4.0 software and were aligned using DNAman™ version 4.1.2.1 software.

Table 6.5. Primers and PCR cycling parameters for the detection of various potential bacterial pathogens

Organism	Primer name	Primer sequence (5'-3')	PCR Cycling Parameters	Gene (Size bp)	References
Legionella spp.	JFP	AGGGTTGATAGGTTAAGAGC	5 min at 95°C; 40 cycles of 94°C for 1 min, 57°C for 1.5 min, 72°C for 1 min	Attachment invasion locus gene (386)	Jonas et al. (1995)
	JRP	CCAACAGCTAGTTGACATCG			
Aeromonas spp.	Aero-F	TGTCGGSGATGACATGGAYGTG	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Aerolysin (720)	Kong et al. (2002)
	Aero-R	CCAGTTCCAGTCCCACCACTTCA			
Shigella spp.	IpaH-F	CCTTGACCGCCTTTCCGATA	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Invasion plasmid Antigen H (606)	Kong et al. (2002)
	IpaH-R	CAGCCACCCTCTGAGGTACT			
Salmonella spp.	IpaB-F	GGACTTTTTAAAAGCGGCGG	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Invasion plasmid Antigen B (314)	Kong et al. (2002)
	IpaB-R	GCCTCTCCCAGAGCCGTCTGG			
Pseudomonas spp.	PA-GS-F	GACGGGTGAGTAATGCCTA	2 min at 95°C; 25 cycles of 94°C for 20 s, 54°C for 20 s, 72°C for 40 s	16S rRNA (618)	Spilker et al. (2004)
	PA-GS-R	CACTGGTGTTCCTTCCTATA			
Yersinia spp.	227Fmod	GTCTGGGCTTTGCTGGTC	5 min at 95°C; 40 cycles of 94°C for 20 s, 60°C for 20 s, 72°C for 15 s	*omp*F (428 - 465)	Stenkova et al. (2008)
	669R	GCGTCGTATTTAGCACCAACG			
Klebsiella spp.	gryA-F	CGCGTACTATACGCCATGAACGTA	3 min at 95°C; 35 cycles of 94°C for 1 min, 50°C for 30 s, 72°C for 30 s	Gyrase A gene (383)	Brisse and Verhoef (2001)
	gyrA-C	ACCGTTGATCACTTCGGTCAGG			

Sequence identification was completed using the National Centre for Biotechnology Information (NCBI) and The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest match of local similarity between isolates

and the international database in GenBank, EMBL, DDBJ and PDB sequence data (Altschul et al., 1990). The sequences of representative isolates, that showed > 97% similarity (< 3% diversity) to organisms was recorded.

6.3 Results and Discussion

6.3.1 Section 1: Preliminary Pasteurization Study

In order to determine which media would be best suited for the monitoring of rainwater treated with various point of use systems, two different media i.e. nutrient agar and R2A agar, generally used in the recovery of heterotrophic bacteria, were compared. Briefly for the laboratory-scale pasteurization experiments, rainwater samples were incubated in a water bath at 50˚C, 55˚C, 60˚C, 65˚C and 72˚C, respectively at five minute intervals (0 min, 5 min, 10 min, 15 min, 20 min, 25 min and 30 min) for each temperature. Untreated and heat treated rainwater samples were then, by means of the spread plate technique, plated onto nutrient agar and R2A agar and incubated at 37˚C (mesophiles) for up to four days. The heat treated samples were also incubated at 10˚C (psychrophiles) and 56˚C (thermophiles) for up to 10 days and four days, respectively, in order to isolate and identify the bacterial groups that were able to withstand extreme temperature conditions.

6.3.1.1 The Recovery of Heterotrophic Bacteria from Untreated and Treated Rainwater Samples Utilising Two Different Media (Nutrient Agar and R2A Agar)

In order to determine which media would be better suited to monitor the efficacy of the solar pasteurization system (Figure 6.2), untreated rainwater samples and rainwater samples heat treated at the various temperatures and different time intervals were spread plated onto nutrient agar (Table 6.7) and R2A agar (Table 6.8) with the plates incubated at 37˚C.

As indicated in Table 6.7, significant differences ($p < 0.05$) in heterotrophic bacteria counts (nutrient agar) were recorded between the untreated rainwater samples and the treated rainwater samples, from the 10 minute time interval at the varying temperature ranges. On average the heterotrophic counts in the untreated rainwater ranged from 2×10^3 to 5×10^3 CFU/mL. While fluctuations in the temperature treated rainwater samples at the different time intervals were noted, on average a two log reduction in the heterotrophic bacteria count was obtained for all the temperatures analysed from the 10 minute time interval. In addition, for the sample treated at 65°C for 25 min, the heterotrophic bacteria count decreased from 2×10^3 CFU/ml (before sample) to zero, with numerous counts decreasing to within the DWAF (1996) standards of < 100 CFU/mL for drinking water.

Table 6.7. The heterotrophic bacteria results for untreated and heat treated rainwater samples spread plated on nutrient agar and incubated at 37°C for 4 days

Temp (˚C)	Heterotrophic Plate Count (CFU/ mL) at time interval (t=x)							
	Before	**0 min**	**5 min**	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
50°C	3×10^3	2×10^2	2×10^2	6×10^1	1×10^2	9×10^1	6×10^1	5×10^1
55°C	4×10^3	7×10^1	4×10^1	4×10^1	5	2×10^1	1×10^1	2×10^1
60°C	2×10^3	3×10^1	1×10^1	5	1×10^1	2×10^1	3×10^1	2×10^1
65°C	2×10^3	1×10^1	2×10^1	5	2×10^1	2×10^1	0	5
72°C	5×10^3	3×10^1	3×10^1	3×10^1	3×10^1	2×10^1	1×10^1	2×10^1

Similarly, significant differences ($p < 0.05$) in the enumeration results for heterotrophic bacteria spread plated onto R2A agar at the different temperature and time intervals were also recorded (Table 6.8). On average the heterotrophic count results obtained for the untreated rainwater samples ranged from 5×10^4 to 2×10^5 CFU/mL. Significant differences ($p < 0.05$) in the heterotrophic plate count at the different treatment temperatures were then recorded from the 10 minute time interval. As indicated in Table 6.9, treatment at 60°C and 72°C from the 5 minute time interval also significantly decreased ($p < 0.05$) the heterotrophic bacteria count to within the DWAF (1996) standards of < 100 CFU/mL for drinking water.

Table 6.8. The heterotrophic bacteria results for untreated and heat treated rainwater samples spread plated on R2A agar and incubated at 37°C for 4 days

Temp (˚C)	Heterotrophic Plate Count (CFU/ mL) at time interval (t=x)							
	Before	**0 min**	**5 min**	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
50°C	1×10^5	2×10^4	6×10^3	9×10^2	7×10^2	3×10^2	6×10^2	4×10^2
55°C	5×10^4	1×10^3	2×10^2	7×10^1	5×10^1	2×10^1	1×10^1	5
60°C	1×10^5	1×10^1	0	0	5	0	5	5
65°C	1×10^5	1×10^1	1×10^1	2×10^1	2×10^1	7×10^1	1×10^1	1×10^1
72°C	2×10^5	2×10^1	0	0	0	5	3×10^1	0

The Thermal Death Time (TDT) is defined as the "shortest time needed to kill all organisms in a microbial suspension at a specific temperature and under defined conditions" (Willey et al., 2008). For the spread plate technique utilising nutrient agar the TDT could not be calculated as growth of heterotrophic bacteria was observed at all temperatures up to t = 30 min, however a count of zero was obtained at the 65°C temperature after the rainwater had been treated for 25 min (Table 6.8). In contrast, while fluctuating results were obtained for the rainwater samples spread plated onto R2A agar after treatment at the varying temperature and time intervals, the TDT was calculated as t = 30 min, at a treatment temperature of 72˚C. In addition, counts of zero were also sporadically obtained at the 60°C and 72°C treatment temperatures from the 5 minute time interval (Table 6.8).

It has previously been noted that the recovering of the level of heterotrophic bacteria gives a good indication of the microbiological quality of water during treatment, storage and distribution of potable water (Carter et al., 2000; DWAF, 1996). Traditionally, to recover heterotrophic bacteria in treated water for potable purposes, plate count agar (PCA) by means of the pour plate method was used (APHA, 1996). It was then proposed that the spread plate method using a low-nutrient media such as Reasoner´s 2A (R2A) agar could be employed (APHA, 1996). For this study a standard minimal media namely, nutrient agar, and a low nutrient media namely, R2A agar were compared for the recovery of heterotrophic bacteria from treated and untreated rainwater.

In the current study the average number of heterotrophic bacteria recovered from the R2A agar (Table 6.8) were significantly higher ($p < 0.05$) when compared to the average HPC CFU/mL recovered from the nutrient agar (Table 6.7). It should be noted that on average a one to two log difference in the heterotrophic counts was obtained when the before rainwater samples were plated onto R2A agar (Table 6.8) versus plating on nutrient agar (Table 6.7). Carter et al. (2000) found that when monitoring a drinking water distribution system in Milford, Ohio (about 30 km east of Cincinnati) the average plate counts were much lower using plate count agar (PCA) and tryptic soy agar with 5% sheep's blood (TSA-SB) compared to average plate counts recovered from R2A agar. Nagarkar et al. (2001) also showed that media modified to enhance the growth of oligotrophs, such as R2A, acquired an increase of about 2 orders of magnitude in the bacteria counts, compared to bacterial counts obtained when samples are cultured with standard minimal media. Moreover, as previously mentioned by Allen et al. (2004), time and temperature of incubation are important variables in determining the HPC bacteria present in an environment. This study therefore, proposes the use of R2A media and incubation at 37˚C for up to four days for the monitoring of microbial contamination of treatment systems in the treatment of rainwater.

6.3.1.2 Bacteria Isolated from Heat Treated Rainwater Samples Incubated at 10˚C and 56˚C on Nutrient Agar and R2A Agar.

Dominant isolate representatives obtained from heat treated samples and incubated at 10˚C and 56˚C on nutrient agar and R2A agar, respectively were selected and identified. The identities of all the bacterial isolates, obtained from the Genbank results (> 98% identity), along with the treatment temperature, plate incubation temperature and time interval of the heat treatment are recorded in Table 6.9. Of the bacteria that were isolated from the heat treated rainwater samples and incubated at 10˚C on R2A agar and nutrient agar, *Flectobacillus* spp. and *Acinetobacter baumannii* were able to withstand heat treatment at 50˚C at t = 0 min and t = 30 min, respectively. *Bacillus aryabhattai* was isolated from rainwater that was heat treated at 55˚C for 30 min, while *Bacillus thuringiensis*

was able to withstand the heat treatment of 60˚C for 30 minutes. Both *Bacillus cereus* and *Bacillus firmus* were isolated from rainwater samples that had been heat treated at 65˚C for 30 minutes.

Of the bacteria that were isolated from the heat treated rainwater samples and incubated at 56˚C on R2A agar and nutrient agar, *Bacillus licheniformis* and *Oceanobacillus* spp. were able to withstand heat treatment at 50˚C at t = 20 min and t = 30 min, respectively. *Bacillus thermolactis* and *Anoxybacillus rupiensis* were also able to withstand the heat treatment of 55˚C for 20 and 30 minutes, respectively. The majority of the bacteria isolated from the heat treated rainwater samples, such as *B. aryabhattai, B. cereus, B. firmus, B. thuringiensis, B. licheniformis, B. thermolactis, Oceanobacillus* spp. and *A. rupiensis,* belonged to the *Bacillaceae* family. *Flectobacillus* spp. and *A. baumannii,* which belong to the *Flexibacteraceae* and *Moraxellaceae* family respectively, were also isolated.

Table 6.9. Bacteria isolated and identified from heat treated samples at different temperatures and for different time intervals

Treatment Temperature (Plate incubation) (°C)	**Time Interval (min)**	**Organism**	**Accession Number**
50 (10)	30	*Acinetobacter baumannii*	AP013357.1
55 (10)	25	*Bacillus aryabhattai*	JX524506.1
65 (10)	30	*Bacillus cereus*	KF601958.1
65 (10)	30	*Bacillus firmus*	KF535122.1
60 (10)	30	*Bacillus thuringiensis*	KF151161.1
50 (10)	0	*Flectobacillus* spp.	AJ011917.1
55 (56)	25	*Anoxybacillus rupiensis*	AM988775.1
50 (56)	20	*Bacillus licheniformis*	HM006901.1
55 (56)	5	*Bacillus thermolactis*	FN666256.1
50 (56)	30	*Oceanobacillus* spp.	HQ316193.1

The rainwater samples were subjected to laboratory scale heat treatment experiments in order to determine which media was suitable for pasteurization experiments and to identify the time and temperature range at which the lowest heterotrophic counts were recorded. A study conducted by La Duc et al. (2007) found similar results when investigating the microbial contamination in clean room environments. With the use of R2A agar physiologically diverse bacteria of the *Bacillaceae* family were identified. It was also noted that in a near neutral environment halotolerant, alkalophilic species such as *Oceanobacillus* and non-spore forming microbes such as *Actinobacter* were also identified.

6.3.2 Section 2: Pilot Scale Pasteurization System

6.3.2.1 Physico Chemical Parameters

The dates the samples were collected from the solar pasteurization system, the temperatures of the untreated (before pasteurization) and treated rainwater samples (after pasteurization) as well as the average ambient temperature are recorded in Table 6.10. Overall an average pH of 6 was measured for all rainwater samples, collected before and after pasteurization. The temperature of the rainwater samples collected from the rainwater harvesting tank ranged from the lowest temperature of 17˚C (11.07.2013) to the highest recorded temperature of 24˚C on two days (26.08.2013 and 02.09.2013). The lowest total rainfall throughout the sampling period was recorded for October (39.6 mm). At the start of the sampling period rainfall was recorded in July 2013 (169.6 mm), which then increased in August 2013 (371.6 mm) and decreased again in September 2013 (177.2 mm).

Table 6.10. The ambient temperature and the temperatures of the untreated and treated rainwater samples on the sampling dates.

Sampling Date	Temperature of untreated rainwater (˚C)	Temperature of treated rainwater (˚C)	Ave. daily ambient temperature (˚C)
11.07.2013	17	56	27.1
22.07.2013	18	57	20.8
30.07.2013	20	65	21.3
19.08.2013	19	55	21.3
20.08.2013	23	81	19.09
26.08.2013	24	78	19.7
02.09.2013	24	64	21.1
06.09.2013	20	81	22.9
06.09.2013	22	91	22.9
10.09.2013	22	66	15.8
10.09.2013	22	74	15.8
11.09.2013	19	72	17
11.09.2013	20	90	17
12.09.2013	21	91	20
09.10.2013	22	73	29.4

In order to monitor the temperature fluctuations of the pasteurized rainwater, the probe of a temperature logger was inserted into the storage tank of the solar pasteurization system and the temperature of the pasteurized rainwater (Figure 6.3) as well as the ambient temperature were monitored for approximately one month (26.07.2013 – 24.08.2013). The direct solar radiation (W/m^2) data was obtained for the same time period as for the temperature logger from the Stellenbosch Weather Services, Engineering Faculty. Readings for both the solar radiation and the temperature were recorded for every 30 minutes. An average of 61˚C and a range of 34˚C (lowest) to 98˚C (highest) was obtained for the temperature of the pasteurized rainwater samples monitored

by the log tagger for the period monitored (Figure 6.3). A significant negative correlation ($R = 0.128$; $p < 0.05$) could be determined between the temperature readings of the stored pasteurized rainwater and the direct solar radiation data obtained, with the direct solar radiation data exhibiting an average of 1.23 W/m^2 and a range of 0 to 741.92 W/m^2. As the direct solar radiation heats the high borosilicate glass collector tubes, the principle of thermo-siphoning heats the rainwater, which loses density, becomes more buoyant, and is then able to move up into the main storage tank. A time delay between the direct solar radiation and final pasteurized water is thus observed, which could explain the significant negative correlation obtained.

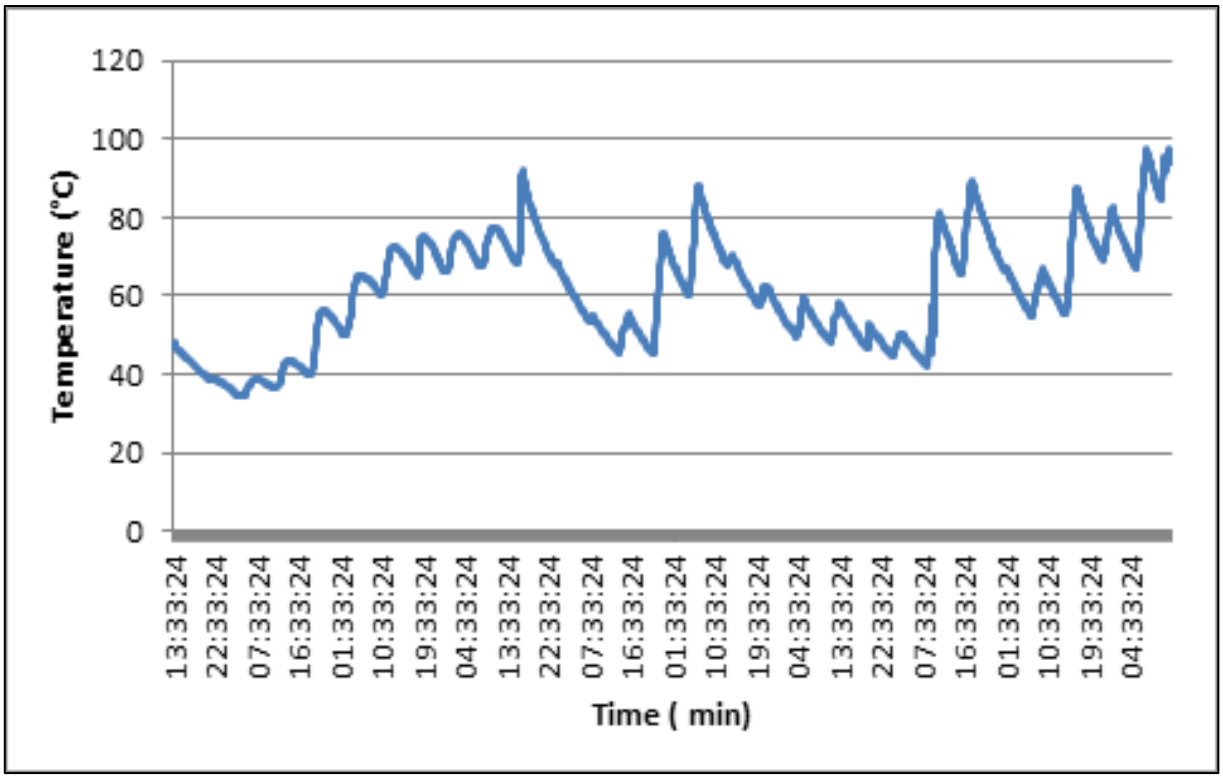

Figure 6.3. Temperatures recorded for the pasteurized rainwater every 30 minutes for approximately one month (26.07.2013 – 24.08.2013).

The ambient temperatures recorded versus the direct solar radiation readings obtained over time are indicated in Figure 6.4. An average of 16˚C and a range of 6˚C (lowest) to 40˚C (highest) was obtained for the ambient temperature using the log tagger (Figure 6.4). A significant positive correlation ($R = 0.74$, $p < 0.05$) was however, observed between the ambient temperature recorded using the log tagger and the direct solar radiation readings obtained from the Stellenbosch Weather Services, which implies that as the direct solar radiation data increased, a similar increase was noted in the ambient temperature.

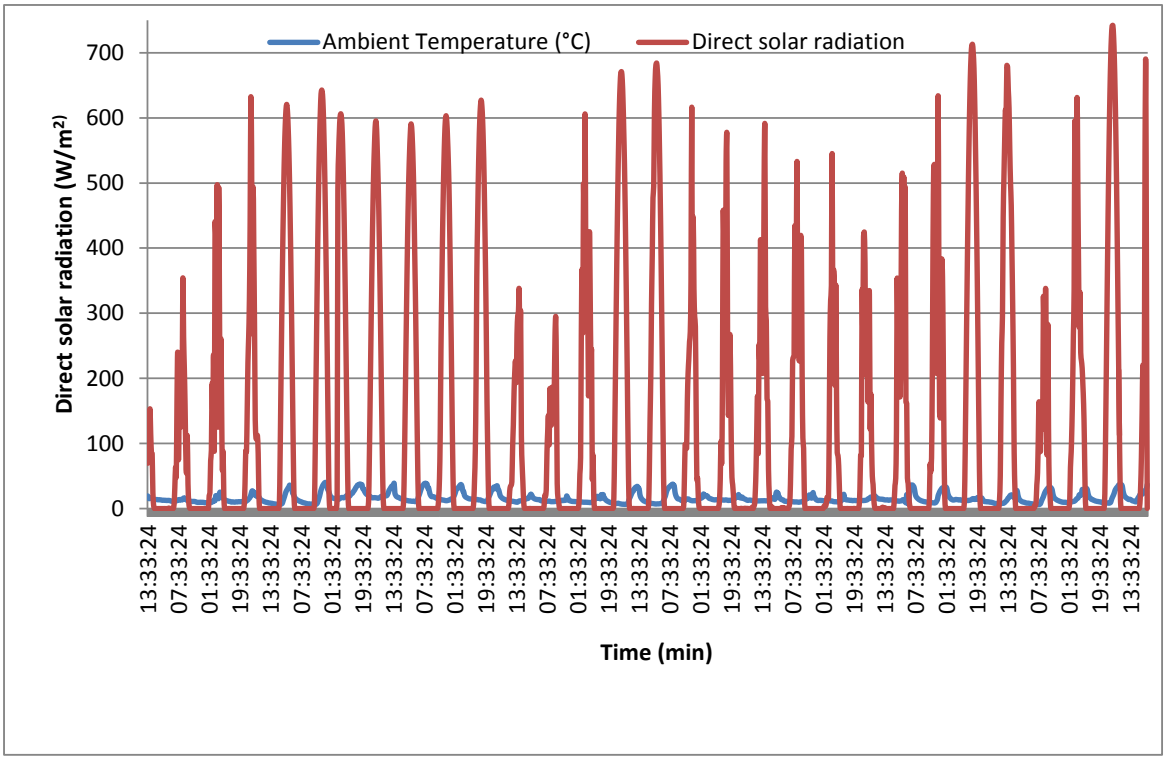

Figure 6.4. Ambient temperatures and the corresponding solar radiation data obtained from the Stellenbosch Weather Services, Engineering Faculty recorded every 30 minutes for approximately one month (26.07.2013 – 24.08.2013).

6.3.2.2 Chemical Analysis

All cations were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of iron, aluminium, lead and nickel as represented in Table 6.11. The DWAF (1996) guidelines stipulate that iron should not exceed 100 µg/L and only one rainwater sample (collected before pasteurization at 55˚C) was within the DWAF (1996) guidelines for iron, with the iron values ranging from the lowest value of 113.4 µg/L (before pasteurization at 65˚C) to the highest value of 441.99 µg/L (before pasteurization at 91˚C) which exceeded the DWAF (1996) guidelines. In addition, two samples were not with the SANS 214 guidelines for iron (200 µg/L), namely the sample taken after pasteurization at 65˚C (218.22 µg/L) and before pasteurization at 91˚C (441.99 µg/L) which was also not within the ADWG of 300 µg/L. No adverse effects among adults have however been noted for consuming high doses of iron. This has been contributed to the physiology of the human body that is able to regulate the absorption of this cation. Iron poisoning has, however, been observed among young children (NHMRC and NRMMC, 2011).

Table 6.11. Cation concentrations obtained from the unpasteurized and duplicate pasteurized rainwater samples collected at various temperatures that were compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 12).

Metal	Before 55˚C	After 55˚C	Before 65˚C	After 65˚C	Before 78˚C	After 78˚C	Before 91˚C	After 91˚C	SANS 241	DWAF	ADWG	WHO
Boron as B (mg/L)	0	0.22	0.00	0.00	0.00	0.26	0.01	0.11	-	-	4	2.4
Calcium as Ca (mg/L)	3.72	6.86	5.03	7.14	4.07	7.00	4.56	4.58	150	200	200	-
Potassium as K (mg/L)	0.28	0.56	0.27	0.49	0.38	0.54	0.31	0.32	50	50	-	-
Magnesium as Mg (mg/L)	0.42	0.95	0.49	0.96	0.44	0.93	0.46	0.50	70	30	200	-
Sodium as Na (mg/L)	3.32	5.58	3.20	5.34	3.33	5.57	2.91	3.14	200	100	180	-
Phosphorus as P (mg/L)	0.03	0.05	0.06	0.05	0.04	0.06	0.03	0.06	-	-	-	-
Silicon as Si (mg/L)	0.32	2.93	0.64	2.58	0.35	3.47	0.55	1.78	-	-	-	-
Aluminium as Al (µg/L)	9.4	61.14	16.0	44.81	15.08	130.98	4.86	48.8	300	150	100	-
Chromium as Cr (µg/L)	0.00	0.39	0.2	0.25	0.18	0.36	0.30	0.3	100	50	50	50
Manganese as Mn (µg/L)	1.20	15.68	0.1	13.67	12.13	13.14	12.77	8.4	100	50	500	-
Iron as Fe (µg/L)	70.59	195.59	113.4	218.22	182.71	179.08	441.99	170.1	200	100	300	-
Cobalt as Co (µg/L)	0.03	0.64	0.0	0.35	0.10	0.50	0.19	0.3	500	-	-	-
Nickel as Ni (µg/L)	0.29	46.43	0.4	22.94	1.04	32.82	1.88	16.0	150	-	20	70
Copper as Cu (µg/L)	4.15	43.57	6.6	19.56	29.34	59.56	23.75	71.7	1000	1000	2000	2000
Zinc as Zn (µg/L)	46.60	338.73	9.1	316.97	57.67	276.62	39.64	171.8	5000	3000	3000	-
Arsenic as As (µg/L)	0.15	0.64	0.4	0.55	0.22	0.41	0.33	0.4	10	10	10	10
Selenium as Se (µg/L)	0.79	1.77	3.7	1.80	0.79	2.36	1.02	0.7	20	20	10	40
Strontium as Sr (mg/L)	31.69	47.16	17.3	43.03	32.12	45.96	39.36	36.7	-	-	-	-
Molybdenum as Mo (µg/L)	0.00	0.00	0.03	0.02	0.00	0.00	0.04	0.0	-	-	50	-
Cadmium as Cd (µg/L)	0.01	0.13	0.0	0.17	0.06	0.19	0.02	0.1	5	5	2	3
Tin as Sn (µg/L)	0.00	0.10	0.1	0.07	0.03	0.07	0.02	0.0	-	-	-	-
Antimony as Sb µg/L	0.02	0.14	0.1	0.20	0.08	0.18	0.14	0.1	-	-	3	20
Barium as Ba (µg/L)	28.93	36.29	11.5	25.93	41.12	35.95	61.79	39.5	-	-	2000	700
Mercury as Hg (µg/L)	0.04	0.03	0.1	0.12	0.01	0.02	0.03	0.0	1	1	1	6
Lead as Pb (µg/L)	0.09	12.81	0.8	3.86	2.46	17.20	1.06	13.2	20	10	10	10

The ADWG (NHMRC and NRMMC, 2011) guidelines stipulate that nickel should not be above 20 µg/L and while all the samples collected before pasteurization and after pasteurization at 91˚C were within the ADWG, samples collected after pasteurization at 55˚C, 65˚C and 78˚C were not within standards and were recorded at 46.43 µg/L, 22.94 µg/L and 32.82 µg/L, respectively. The concentration of nickel in all the rainwater samples, were however, within the stipulated standards of the SANS 241 (2005) and WHO (2011) of 150 µg/L and 70 µg/L, respectively. Nickel is distributed by the blood in the body, with the main excretion route via the urinary tract. The toxicity exhibited by nickel compounds is dependent on many factors such as the chemical species, their physical form, their concentration and whether the individual is exposed to nickel by ingestion, inhalation or dermal interaction (Christensen and Lagesson, 1981).

The average concentration of the aluminium in the rainwater samples was within the drinking water guidelines as stipulated by SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011). The average concentration of rainwater samples collected after pasteurization at 78˚C, however, exceeded the ADWG (NHMRC and NRMMC, 2011) of 100 µg/L, with an average concentration of 130.98 µg/L recorded. Although the effect of aluminium on human health needs to be clarified, continuous exposure of humans to high concentrations of aluminium has been implicated in chronic neurological disorders such as Alzheimer's disease, Parkinsonism dementia (PD) and amyotrophic lateral sclerosis (ALS). It is also important to take into consideration that at a neutral pH aluminium is non-toxic and is not an essential nutrient for humans (DWAF, 1996; Perl, 1985).

The concentration of lead in drinking water should not exceed 10 µg/L according to DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011). However three samples collected after pasteurization at 55˚C, 78˚C and 91˚C exceeded these guidelines with average concentrations of 12.81, 17.20 and 13.2 µg/L recorded in the respective rainwater samples. These values were however, still within the SANS 241 (2005) guideline of 20 µg/L. Lead is a powerful and persistent neurotoxicant and the effects of lead poisoning range from death to impaired cognitive and behavioral development that can have long term detrimental consequences for children (Lidsky and Schneider, 2003).

The noteworthy changes in the concentrations of the cations present in the rainwater samples before and after treatment at 55˚C will be discussed first. The increase in manganese, cobalt, nickel, copper, lead and zinc was significant (more than a 10 fold increase) as concentrations

before and after pasteurization ranged from 1.20 µg/L to an average of 15.68 µg/L, from 0.03 µg/L to an average of 0.64 µg/L, from 0.29 µg/L to an average of 46.43 µg/L, from 4.15 µg/L to an average of 43.57 µg/L, from 0.09 to an average of 12.81 µg/L and from 46.60 µg/L to an average of 338.73 µg/L, respectively. The concentration of tin increased after treatment from 0 µg/L to an average of 0.1 µg/L and mercury decreased from 0.04 µg/L to an average of 0.03 µg/L, these changes were, however, negligible (0.1 µg/L). All the other cations monitored also increased after treatment at 55˚C, with molybdenum being the only exception as this cation was not detected in either the before or after treatment samples.

While increases for many of cations monitored before and after treatment at 65˚C were recorded, the only significant increase (more than a 10 fold increase) for the rainwater samples treated at 65˚C was observed for zinc. The concentration of zinc increased from 9.1 µg/L in the before sample to an average of 316.87 µg/L in the after 65˚C treated sample. In addition, decreases were observed after treatment at 65˚C for phosphorous, selenium, molybdenum and tin, with concentrations before and after pasteurization ranging from 0.06 µg/L to an average of 0.05 µg/L, from 3.7 µg/L to an average of 1.8 µg/L, from 0.03 µg/L to an average of 0.02 µg/L and from 0.1 µg/L to an average of 0.07 µg/L, respectively. Boron was the only cation which was not detected in either the before or after treatment samples.

Nickel was the only cation that had a significant increase (more than 10 fold increase) in concentration after treatment at 78˚C with a concentration of 1.04 µg/L observed before treatment and an average concentration of 32.82 µg/L observed after treatment. The concentrations of iron and barium decreased after treatment at 78˚C with before and after concentrations ranging from 182.71 µg/L to an average of 179.08 µg/L and from 41.12 µg/L to an average of 35.95 µg/L, respectively. All the other cations monitored also increased after treatment at 78˚C, however the increase was not significant. Molybdenum was not detected in either the before or after treatment samples.

The concentration of aluminium and lead increased significantly (more than 10 fold increase) after treatment at 91˚C with the before and after concentrations ranging from 4.86 µg/L to an average of 48.8 µg/L and from 1.06 µg/L to an average of 13.20 µg/L, respectively. In contrast, the concentration of manganese, iron, barium and mercury decreased after treatment at 91˚C with before and after concentrations ranging from 12.77 µg/L to an average of 8.4 µg/L, from 441.99 µg/L to an average of 170.1 µg/L, from 61.79 µg/L to an average of

39.5 µg/L and from 0.03 µg/L to an average of 0.0 µg/L, respectively. All the other cations monitored also increased in concentration after treatment at 91˚C, however the increase in concentration was not significant.

Iron, aluminium, lead and nickel were shown to be present at higher concentrations in the rainwater samples collected from the pasteurized solar system (at varying temperatures) than in the unpasteurized samples stored in the rainwater tank. The rainwater is in direct contact with borosilicate glass collector tubes and the main stainless steel storage tank. Researchers have shown that during simulated cooking process, nickel leaches from stainless steel cooking ware into food (Kamerud et al., 2013). In a study conducted by Semwal et al. (2006) aluminium also leached from stainless steel cooking utensils during food preparation. Therefore it is hypothesised that the iron, aluminium, lead and nickel were leached from the stainless steel storage tank into the rainwater during the current study.

All anions were within drinking water guidelines according to SANS 241 (2005), DWAF, (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) and are represented in Table 6.12. Sulphate concentrations after pasteurization at 55˚C and 65˚C, increased from 1.1 mg/L to an average of 5.35 mg/L and from 1.6 mg/L to an average of 5.7 mg/L, respectively. At higher pasteurization temperatures of 78˚C and 91 ˚C the concentrations of sulphate increased from 1.2 mg/L to an average of 3.5 mg/L and from 1.6 mg/L to an average of 1.95 mg/L, respectively. Chloride concentrations increased after pasteurization at 55˚C and 65˚C from 7.2 mg/L to an average of 12 mg/L and from not being detected to an average of 11 mg/L, respectively. A decrease in the concentration of chloride was however, observed after pasteurization at 78˚C from 7.2 mg/L to an average of 3.1 mg/L. At 91˚C no changes in the concentration of chloride was observed with 5.9 mg/L of chloride observed in both the before and after pasteurization rainwater sample. In addition, no significant change in concentrations was observed for nitrate and nitrite, phosphate and fluoride in the rainwater samples after pasteurization for all temperatures (55 to 91˚C).

Table 6.12. Anions concentrations obtained from the unpasteurized and duplicate pasteurized rainwater samples collected at each temperature that were compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 12).

Anions	**Before 55˚C**	**After 55˚C**	**Before 65˚C**	**After 65˚C**	**Before 78˚C**	**After 78˚C**	**Before 91˚C**	**After 91˚C**	**SANS 241**	**DWAF**	**ADWG**	**WHO, (2011)**
Sulphate as SO_4 (mg/L)	1.1	5.35	1.6	5.7	1.2	3.5	1.6	1.95	200	100	250	-
Chloride as Cl^- (mg/L)	7.2	12	ND	11	7.2	3.1	5.9	5.9	400	200	250	-
Nitrate Nitrite as NO_3 and NO_2 (mg/L)	0.1	0.1	ND	0.1	0.1	0.1	0	0	10	6	50	50
Phosphate as PO_4 (mg/L)	0.05	0.05	ND	ND	0.05	0.1	0	0	-	-	-	-
Fluoride as F (mg/L)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	1	1	1.5	1.5

ND – not detected

6.3.2.3 Indicator Bacteria Detected in Untreated and Solar Pasteurized Water Samples

Untreated (1 L) and duplicate treated rainwater samples (2 x 1 L) were collected from a solar pasteurization system at various temperatures. The lowest temperature range included in this study was 55 to 57˚C and the highest range was 90 to 91˚C. Total coliforms, *E. coli* and HPC were enumerated for all treated and untreated rainwater samples at the varying temperature ranges (Table 6.13). In addition, heterotrophic bacteria were enumerated in stored solar pasteurized treated rainwater samples (for every temperature range) every day for a week in order to determine the maximum storage time allowed for the pasteurized rainwater before the recovery of heterotrophic bacteria was observed (Table 6.13).

Table 6.13. Indicator bacteria enumerated from pasteurized rainwater samples at various temperatures and the amount of time allowed for storage of the heat treated rainwater samples

Pasteurization Temperature (˚C)	Indicator	Untreated Sample (Ave. CFU/100 mL)	First 1 L Treated (Ave. CFU/100 mL)	Second 1 L Treated (Ave. CFU/100 mL)	Log Reduction	Reduction (%)	Storage (Days)
55 -57	Total Coliforms	7×10^2	0	0	2	100	0
	E. coli	4×10^1	0	0	1	100	
	HPC	2×10^7	6×10^5	5×10^5	2	97	
64 - 66	Total Coliforms	7×10^2	0	0	2	100	0
	E. coli	2×10^1	0	0	1	100	
	HPC	3×10^6	4×10^5	5×10^5	1	86	
72 - 74	Total Coliforms	6×10^2	0	0	2	100	2
	E. coli	1	0	0	0	100	
	HPC	3×10^6	0	0	6	100	
78 - 81	Total Coliforms	6×10^2	0	0	2	100	7
	E. coli	4×10^1	0	0	1	100	
	HPC	4×10^6	0	0	6	100	
90 - 91	Total Coliforms	3×10^3	0	0	3	100	7
	E. coli	4×10^1	0	0	1	100	
	HPC	4×10^6	0	0	6	100	

At each pasteurization temperature an untreated and duplicate treated rainwater samples were collected and analysed for the presence of indicator bacteria. On average, in comparison to the untreated rainwater, the rainwater samples pasteurized at 55˚C, 56˚C and 57˚C yielded a two log

reduction in the average total coliforms and a one log reduction in the average *E. coli* counts, with no total coliforms and *E. coli* detected after treatment (Table 6.13). Similar results were obtained for the rainwater samples pasteurized at 64˚C, 65˚C and 66˚C; 72˚C, 73˚C and 74˚C; and 78˚C, 81˚C and 81(2^{nd})˚C, with the exception of *E. coli* numbers for the 72 to 74ºC temperature range where no log reduction was observed. In addition, comparison between the before treatment rainwater sample (3×10^3 CFU/mL) and the rainwater solar pasteurized at 90˚C, 91˚C and 91(2^{nd})˚C yielded a three log reduction in the average total coliforms, with no total coliforms detected in the treated samples. The initial *E. coli* count of 4×10^1 CFU/mL was also decreased to zero after treatment at 90˚C, 91˚C and 91(2^{nd})˚C.

Comparison between the heterotrophic plate counts on R2A for the untreated rainwater samples (2×10^7 CFU/mL) and the rainwater samples pasteurized at 55˚C, 56˚C and 57˚C showed a 2 log reduction in average heterotrophic bacteria counts. In addition, the average heterotrophic plate count of 3×10^6 CFU/mL recorded in the rainwater sample before treatment at temperatures 64˚C, 65˚C and 66˚C, was reduced by one log to 4×10^5 and 5×10^5 CFU/mL in the duplicate solar pasteurized treated rainwater samples (Table 6.13). Rainwater samples pasteurized at 72˚C, 73˚C and 74˚C; 78˚C, 81˚C and 81(2^{nd})˚C; and 90˚C, 91˚C and 91(2^{nd})˚C, showed a significant six log reduction in the average heterotrophic bacterial values, in comparison to the before treatment rainwater heterotrophic counts, with no heterotrophic bacteria detected in the pasteurized rainwater samples (Table 6.13).

Overall, all total coliforms and *E. coli* numbers enumerated after the pasteurization treatment ranging from temperatures of 55 to 57˚C up to 90 to 91˚C were reduced to zero and were within the DWAF (1996) guidelines. However the HPC numbers were above the DWAF (1996) guidelines for the temperature ranges of 55 to 57˚C (average 5.5×10^5 CFU/ mL) and 64 to 66˚C (average 4.5×10^5 CFU/ mL), with no HPC numbers obtained after solar pasteurization at the temperature range of 72˚C to 91˚C.

To monitor the maximum storage time of the rainwater pasteurized at the various temperature ranges as outlined in Table 6.3, Section 6.2.1, samples were stored at room temperature for up to one week. The re-growth of heterotrophic bacteria after pasteurization at the various temperatures was observed and heterotrophic counts were enumerated daily. As HPC numbers were above the DWAF (1996) guidelines for the temperature ranges of 55 – 57˚C (average 5.5×10^5 CFU/mL) and 64 to 66˚C (average 4.5×10^5 CFU/ mL) the regrowth of HPC was observed after one day. In contrast the rainwater treated at higher solar pasteurization temperatures could be stored for approximately two days (72 to 74˚C) and up to one week (78 to 81˚C, 90 to 91˚C). In summary the

storage time allowed before HPC numbers grew to above the DWAF (1996) guidelines ranged from no storage allowed, (55 to 57˚C; 64 to 66˚C) to two days of storage (72 to 74˚C) and one week of storage if the water was pasteurized at the higher temperatures (78 to 81˚C, 90 to 91˚C). The *D* value has been defined as the time required in reducing a bacterial population by 90% or a 1 log reduction (Prescott et al., 1993) and is used by a number of studies in determining the time required to reduce bacterial numbers (Juneja et al., 2001; Spinks et al., 2006). However, *D* values could not be established for the heat treatment of total coliforms, *E. coli* and HPC bacteria found in rainwater in this study as the solar pasteurization is a continuous flow system and the number of inactive bacterial cells does not necessarily represent the number of active bacterial cells entering the storage tank of the pasteurization system. Spinks et al. (2006) have described the limitations of using the *D* value thermal inactivation data as it is assumed that there is a constant reduction rate over time. It is therefore suggested that in order to evaluate the efficacy of a heat treatment system, conclusions should not be made by relying solely on the *D* value. However, this study showed that heterotrophic bacteria were reduced by an average of 85% at temperatures ranging from 55˚C to 65˚C and from temperatures of 72˚C onward a 100% reduction in heterotrophic bacteria was observed. However, total coliform and *E. coli* numbers were reduced by 100% with no total coliforms or *E. coli* observed from 55˚C and higher. Spinks et al. (2006) also suggested that water temperatures should reach between 55 and 65˚C in order to eliminate enteric pathogenic bacteria. Other studies have suggested that temperatures below boiling greatly reduce bacterial numbers from rainwater samples resulting in water quality that is within the Australian Drinking Water Guidelines (Coombes et al., 1999; 2003; Lye, 1991). However, it has been suggested that *Enterococcus faecalis* instead of *E. coli* should be used as an indicator organism for the monitoring of hot water quality as *E. faecalis* demonstrated the greatest heat resistance at 55˚C (Spinks et al., 2006).

6.3.2.4 The Detection of Coliphages in Harvested Rainwater

As indicated in Chapter 5, the number of coliphages present in the harvested rainwater samples (unpasteurized and pasteurized at temperatures mentioned in Section 6.2.1, Table 6.3) were below the detection limit. However, when unpasteurized rainwater (500 mL) was spiked with 10 mL of *E. coli* ATCC 13706 (that had been incubated at 37˚C until log phase reached) 1 PFU/ mL was detected. These results were thus not significant and overall coliphages could not be detected in the rainwater samples before and after pasteurization.

6.3.2.5 Genus Specific PCR Detection of Bacteria Commonly Identified in Harvested Rainwater

Untreated rainwater samples as well as the rainwater samples treated at various temperatures (outlined in Section 6.2.1, Table 6.3) by solar pasteurization, were screened using PCR assays for

the detection of various pathogenic bacteria commonly associated with harvested rainwater. The results of the PCR assays are presented in Appendix A, Table 6.1. The percentages of the various bacterial genera present in the untreated rainwater and two pasteurized rainwater samples, along with the highest pasteurization temperature where the PCR assays tested positive, are summarised in Table 6.14.

Table 6.14. The percentage of untreated rainwater and duplicate pasteurized rainwater samples that tested positive for various bacterial genera and the highest pasteurization temperature where the PCR assays tested positive

Organism	Rainwater Samples (%)		Highest Pasteurization Temperature (˚C)
	Untreated	Pasteurized (n = 30)	
Aeromonas spp.	20	0	56
Klebsiella spp.	47	17	65
Legionella spp.	87	100	91
Pseudomonas spp.	67	47	91
Salmonella spp.	0	0	NA
Shigella spp.	7	3	55
Yersinia spp.	27	13	78

No *Salmonella* spp. were detected with the use of PCR based assays throughout the study in the untreated and pasteurized rainwater samples at the various temperature ranges (Table 6.14). In addition, while PCR assays confirmed the presence of *Aeromonas* spp. (GenBank accession no. CP005966.1) in the unpasteurized rainwater samples collected before treatment at 56˚C, 57°C and 65°C, no *Aeromonas* spp. were detected in the solar pasteurized rainwater samples and throughout the remainder of the study period (Table 6.1, Appendix A). *Shigella* spp. (GenBank accession no. HE616529.1) were also only detected twice throughout the study period in a duplicate rainwater sample pasteurized at 55°C and in an untreated rainwater sample collected before pasteurization at 56°C. Similarly, *Yersinia* spp. were present in a duplicate rainwater sample pasteurized at 55°C and in both rainwater samples collected after treatment at 78°C (Table 6.1, Appendix A). For each temperature, samples are collected before treatment from the rainwater tank, with solar treated samples collected directly from the pasteurization unit. This could explain why *Shigella* spp. and *Yersinia* spp., amongst others, were present in the after treatment sample and not in the initial sample collected directly from the rainwater tank. *Yersinia* spp. were also detected sporadically throughout the sampling period in untreated rainwater samples collected before treatment at 57°C, 73°C and 81°C, with no *Yersinia* spp. detected in the samples solar pasteurized at these respective temperatures. However, *Yersinia* spp. were detected in the untreated rainwater sample collected

before solar pasteurization at 65°C as well as in a duplicate rainwater sample pasteurized at this temperature (Table 6.1, Appendix A).

Klebsiella spp. (GenBank accession no. AF303617.1) were also only detected sporadically throughout the study period at the varying temperature ranges, with this organism present only in the unpasteurized rainwater samples collected for the temperatures 55°C, 56°C, 81°C and 91°C. In addition, *Klebsiella* spp. were detected using PCR assays in the before and after solar pasteurization samples collected at 57°C and 65°C, where it was present in only one after treatment sample, and 64°C, where it was present in the before and both samples analysed after solar pasteurization at this respective temperature. Similar to the results obtained for *Shigella* and *Yersinia* spp., *Klebsiella* spp. were also detected in a duplicate rainwater sample pasteurized at 73°C, while it was not detected in the before treatment rainwater sample collected directly from the rainwater tank.

The frequency of detection for *Pseudomonas* spp. and *Legionella* spp. were highest throughout the study period (Figure 6.5; Table 6.1, Appendix A), with *Pseudomonas* spp. detected in 67% of the untreated rainwater samples and 47% of the solar pasteurized samples collected at various temperature ranges. Overall *Legionella* spp. were the most persistent organisms and were present in all the rainwater samples, unpasteurized and pasteurized (for all the temperature ranges) collected throughout the study period as indicated in Table 6.1, Appendix A.

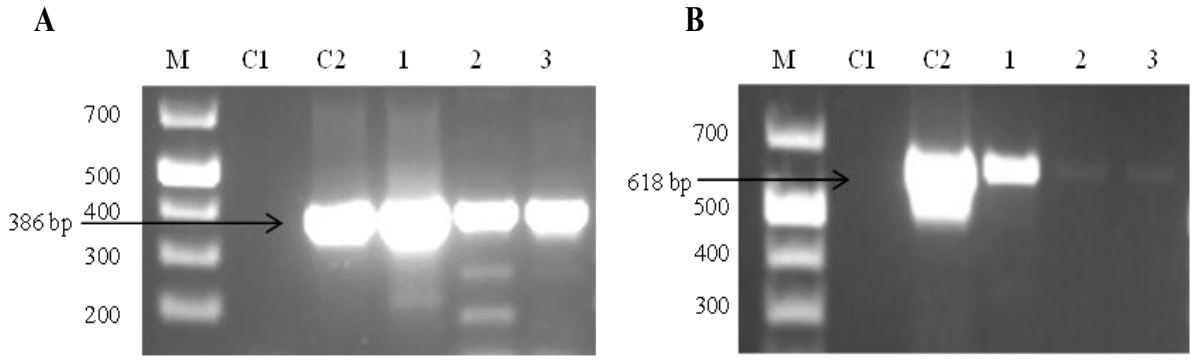

Figure 6.5. Agarose gel electrophoresis (1.2%) of PCR assays conducted to detect the presence of *Legionella* spp. (A) and *Pseudomonas* spp. (B). Lane M represents a 1 kb ladder which was used as a molecular size marker with the sizes indicated in base pairs. Lane C1: the respective negative controls. Lane C2: the respective positive controls. Lane 1: Unpasteurized rainwater sample. Lanes 2 and 3: the duplicate rainwater samples, pasteurized at 55˚C.

The PCR based assays utilising genus specific primers thus detected the presence of *Legionella* spp., *Yersinia* spp., and *Pseudomonas* spp. at the pasteurization temperature greater than 78˚C. No literature has previously reported the presence of viable planktonic *Legionella*, *Pseudomonas*, or *Yersinia* species cable of surviving treatment temperatures of approximately 78˚C and higher. In

this study however organisms identified at temperatures greater than 78˚C according to Genbank (> 97% identity) included uncultured *Legionella* (GenBank accession no. KC209485.1, AB858005.1, KC209446.1) and *Yersinia enterocolitica* (GenBank accession no. HM142628.1). Results also confirmed the presence of uncultured *Pseudomonas* sp. (GenBank accession no. JX279939.1), *Pseudomonas stutzeri* (GenBank accession no. KF260975.1), *Pseudomonas* sp. (GenBank accession no. KF561877.1) at temperatures greater than 78˚C. The viability of these organisms however, is not verified and of the many pitfalls associated with PCR detection methods, PCR based assays cannot distinguish between viable and non-viable pathogenic organisms (Ahmed et al., 2013). However, it is hypothesised that the presence of these organisms at such high pasteurization temperatures could be due to two possible scenarios, either the PCR based assays were detecting non-viable cells, or the bacteria were ingested by protozoa. Protists, especially amoebae, have been described by Bichai et al. (2008) as "the Trojan Horse of microorganisms" as protozoa are cable of heterotrophic feeding. Amoebae have two stages of development generally, known as the trophozite and the cyst which, because of the two layers that surround it, is cable of surviving treatments such as chlorination and temperatures of between -20˚C and +42˚C (Greub and Raoult, 2004). *Legionella* spp. and amoeba have also been detected simultaneously in rainwater sources (Lye, 2002). As amoeba, like many protists, feed mainly on bacteria, and most bacteria are able to survive after being ingested, studies have shown that various species of *Legionella* are able to remain viable after being ingested by species of *Acanthamoebae*. For example, *Legionella erythra* and *Legionella pneumophila*, ingested by *Acanthamoeba castellanii* and IA (an environmental thermotolerant *Acanthamoebae* isolate) increased by 1 – 2 logs after being treated at temperatures varying from 40 to 80˚C (Storey et al., 2004). Other bacteria that have been seen to be ingested by *A. castellanii* include, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Shigella sonnei* and *E. coli* (King et al., 1988). This hypothesis will however, have to be investigated in future research projects. In addition, viability assays will have to be conducted on the solar pasteurized samples.

6.3.2.6 Determining the Maximum Volume of Rainwater Harvested from the Solar Pasteurization System

The volume of pasteurized rainwater that was harvested from the 100 L storage tank of the solar pasteurization system at various temperature ranges is summarised in Table 6.15. As the storage tank of the solar pasteurization system was drained, the temperature of the pasteurized rainwater decreased by approximately 4˚C in an average of 21:47 minutes. An average of 64.4 L could also be harvested before the flow rate decreased and pasteurized rainwater could no longer be collected form the solar system.

Table 6.15. The volume of pasteurized rainwater harvested at various temperatures

Temperature Range (˚C)	Pasteurized Rainwater Harvested (L)	L/m^2 for each kWh of Incident Solar Energy
89 - 93	68	0.99
87 – 90	65	1.2
71 - 73	63	11.9
93 - 97	63	0.6
56 - 58	63	61.8

In addition, on two occasions, the temperature of the pasteurized rainwater in the storage tank of the solar system was monitored while the water was drained from the system. When the temperature of the system reached approximately 93˚C, the storage tank was drained [Figure 6.6 (green arrow)] and after approximately 4.5 hours the system had stabilised (the storage tank had been re-filled with rainwater) and the temperature of the rainwater started to increase again to approximately 54˚C. On the second occasion, when the temperature of the storage tank had reached approximately 50˚C, the storage tank was again drained (Figure 6.6 (red arrow)) and approximately 7.75 hours later the system had stabilised (the storage tank had been re-filled with rainwater) and the temperature of the rainwater started to increase again to approximately 30˚C.

The direct solar radiation data showed an average of 1.82 W/m^2, 2.28 W/m^2, 23.79 W/m^2, 1.27 W/m^2 and 122.6 W/m^2 for the corresponding pasteurized water temperature of 89˚C, 87˚C, 71˚C, 93˚C and 56˚C, respectively (see Section 6.3.2.1, Figure 6.4) and it was then determined that the system could produce an average of 61.8 L/m^2 for each kWh of incident solar energy, for lower temperatures ranging from 56 to 58˚C and at higher temperatures (93 to 97˚C), the system could produce approximately 0.6 L/m^2 for each kWh of incident solar. El-Ghetany and Dayem (2010) found that a solar system could produce 171 L/m^2 daily at 60˚C which also decreased to 39 L/m^2 at higher temperatures of 90˚C. Other studies were able to harvest 3.61 L/m^2 for each kWh of incident solar at 80˚C (Bansal et al., 1988), 2.8 L/m^2 for each kWh of incident solar at 70˚C (El-Ghetany and El-Seesy, 2005) and 1.1 L/m^2 for each kWh of incident solar at 90˚C (El-Ghetany and Dayem, 2010).

It was reported that in Southern Africa, households generally use water for drinking, cooking and hygiene purposes. Studies indicated that the average household requires at least 20 L of potable water per day, of which 50% of the water is utilised for personal hygiene (Nieuwoudt and Mathews, 2005). Reiff et al. (1996) found that in Latin America average families of five members required approximately between 40 L and 60 L of potable water. Taylor (2001) found that approximately 50% of people living around and in Pretoria did not have access to running water in their houses. Le Roux (2003) showed that 60% of the rural community in Mabedlane, KwaZulu – Natal, collected

their water from the Umgeni River although central taps connected to the main municipal lines where available. It is thus estimated that the pasteurization system used in the current study should provide an adequate volume of treated rainwater for drinking and domestic purposes at the average sized household level (up to four people).

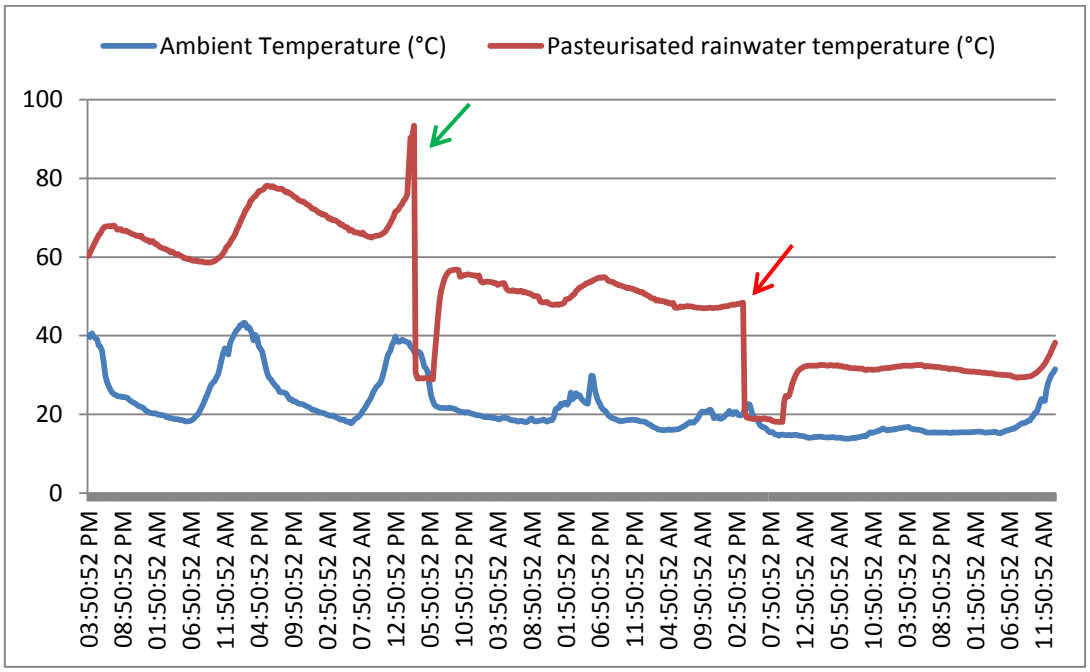

Figure 6.6. The temperature of the rainwater inside the storage tank over a two week period, monitoring the temperature changes due to the system being drained sporadically (five times). The temperature logger was adjusted to record readings every 15 minutes. Arrows indicate where the storage tank was drained of pasteurized rainwater.

6.4 Conclusions

The first phase of the study was aimed at assessing the efficiency of different pasteurization temperatures (laboratory scale treatment of rainwater samples) and determining the optimum culture media to enumerate heterotrophic bacteria, which served as a measure of disinfection efficiency, at the laboratory scale level. While preliminary pasteurization results indicated that significant differences ($p < 0.05$) in the heterotrophic plate count at the different treatment temperatures were recorded from the 10 minute time interval on both nutrient agar and R2A (Tables 6.7 and 6.8) and that treatment at 65°C to 72°C also significantly decreased the heterotrophic bacteria count to within the DWAF (1996) standards of < 100 CFU/mL for drinking water, the thermal death time was calculated as t = 30 min, at a treatment temperature of 72˚C (from results obtained for R2A agar). In addition, based on the results obtained, R2A agar was the most suited growth media for enumerating the recovery of heterotrophic bacteria from heat treated rainwater samples and this media was thus utilised to assess the efficiency of the solar pasteurization system

in the on-site pilot scale study. Dominant bacterial isolate representatives obtained from heat treated samples and incubated at 10˚C and 56˚C on nutrient agar and R2A agar, respectively were also selected and identified. The majority of the bacteria isolated from the heat treated rainwater samples, such as *B. aryabhattai, B. cereus, B. firmus, B. thuringiensis, B. licheniformis, B. thermolactis, Oceanobacillus* spp. and *A. rupiensis,* belonged to the *Bacillaceae* family.

In the second phase of this study a heating based disinfection system, namely a closed coupled system (pilot scale treatment of rainwater samples directly from a DRWH tank) was analysed for harvested rainwater treatment. The microbial parameters that were investigated during the pilot scale study included the enumeration of faecal and total coliforms, *Escherichia coli* and heterotrophic bacteria. In addition, the treated and untreated rainwater samples of the pilot scale study were screened for the presence of selected pathogenic bacteria using molecular techniques. The chemical parameters that were investigated during the pilot scale study included the concentration of metal ions, anions and cations present in the treated and untreated harvested rainwater samples.

Chemical analysis indicated that all cations and anions present in both the unpasteurized and pasteurized water at all the varying temperature ranges were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of iron, aluminium, lead and nickel. These four cations were shown to increase significantly ($p < 0.05$) after pasteurization at various temperatures and it is hypothesised that these elements could have leached from the stainless steel storage tanks of the pasteurization system. A company in Somerset West, Crest, is currently developing a solar pasteurization system with a storage tank that will be manufactured from Safrene^{TM}, which is a high density based polyethylene. It is therefore recommended that the storage tank of the pasteurization system be manufactured from an alternative material, such as a high grade polymeric material, which is able to withstand the high temperatures yet will not negatively influence the quality of harvested rainwater.

While heterotrophic bacteria still persisted at the 55 to 57ºC and 64 to 66ºC temperature ranges, total coliform, *E. coli* and heterotrophic bacteria counts were reduced to zero (100%) in the rainwater samples pasteurized at the 72 to 74˚C, 78 to 81˚C, and 90˚C to 91˚C temperature ranges (Table 6.13). It is therefore recommended that the minimum temperature required to treat harvested rainwater should be above 72ºC in order to utilise rainwater for drinking and domestic purposes. In addition, at the higher temperature ranges (78 to 81˚C, and 90˚C to 91˚C) pasteurized rainwater could safely be stored for to up to 7 days before bacterial re-growth occurred. The number of

coliphages present in the unpasteurized and pasteurized harvested rainwater samples, were also below the detection limit throughout the study period.

Based on genus specific PCR analysis, utilised to screen pasteurized rainwater samples for the presence of pathogenic bacteria that have previously been detected in harvested rainwater (Chapter 2), no *Salmonella* spp. were detected in any of the rainwater samples analysed. However, while *Aeromonas* spp., *Klebsiella* spp., and *Shigella* spp. were not detected in the rainwater samples solar pasteurized at temperatures of higher than 72ºC, *Yersinia* spp., *Legionella* spp. and *Pseudomonas* spp. were detected in the rainwater samples pasteurized at the temperature ranges of 72 to 74˚C and 78 to 81˚C, while *Legionella* spp. and *Pseudomonas* spp. were still detected at the temperature range of 90 to 91ºC.

Of particular concern is the presence of *Legionella* spp. at such high temperatures. Previously this pathogen has been isolated from shower heads, whirlpools spas, cooling towers, air conditioning systems and humidifiers. Further studies should include the detection of viable *Legionella* cells as it is possible that the PCR assays used in this study detected the DNA of non-viable bacteria cells, which is a major drawback when utilising PCR analysis. As previously noted, misleading PCR results such as false positives or negatives could wrongfully deem the rainwater inadequate and this could lead to the installation of unnecessary expensive treatment systems (Ahmed et al., 2013). For this reason future studies will incorporate whole sample quantitative analysis, such as quantitative PCR (qPCR) and flow cytometry analysis using DNA probes specific for certain bacteria, in order to confirm the presence of viable pathogenic organisms within rainwater samples pasteurized at temperatures of greater than 72ºC.

The average temperature readings recorded during July to August (winter period) for the rainwater in the pasteurization system was 61ºC with a maximum temperature of 98ºC and a minimum temperature of 34ºC recorded. This data is important when determining the volume of rainwater that can be treated with the pasteurization system. It was determined that the solar pasteurization system could produce an average of 61.8 L/m^2 for each kWh of incident solar energy, for lower temperatures ranging from 56 - 58˚C and at higher temperatures (93 - 97˚C), the system could produce approximately 0.6 L/m^2 for each kWh of incident solar. Therefore throughout the year, including the winter months, large volumes of rainwater can be treated efficiently utilising solar pasteurization to produce water for drinking and domestic purposes.

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Chapter 7: General Conclusions and Recommendations

(Chapter 7 is compiled in the format of the Water Research Commission report and UK spelling is employed)

7.1 General Conclusions and Recommendations

In South Africa, harvested rainwater is generally used for domestic purposes, irrigation purposes and other small scale activities (Mwenge Kahinda et al., 2007). Countries such as Australia (Ahmed et al., 2012), Bermuda (Lévesque et al., 2009), New Zealand (Simmons et al., 2001) and Greece (Sazakli et al., 2007) have implemented the use of rainwater harvesting and have indicated that the quality of rainwater is within the respective drinking water guidelines for chemical parameters, however the microbiological quality of harvested rainwater is of concern. To date, the quality of harvested rainwater in South Africa has not been extensively monitored with one study completed by Nevondo and Cloete (1999) where the microbial and chemical parameters were monitored in one rainwater source. So far treatment of harvested rainwater has involved boiling and a few cases have been documented where households were drinking harvested rainwater after treating the rainwater with drops of bleach (Duncker, 2000). The purpose of this study was thus to monitor the microbial and chemical quality of harvested rainwater in Kleinmond, a small coastal town located in the Western Cape of South Africa. The second aim of the study was then to design and monitor the efficacy of various treatment systems in reducing the microbiological load of harvested rainwater. The results, of the monitoring as well as the treatment phase, were compared to the South African Water Quality Guidelines (DWAF, 1996), the South African National Standards for Drinking Water (SANS, 2005), the World Health Organisation (WHO, 2011) and the Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011) to assess the quality of harvested rainwater.

In the first phase of the project (Chapter two), the results obtained for the chemical analysis performed for the 29 rainwater tanks in the Kleinmond Housing Scheme, for the sampling period March to August 2012, indicated that the rainwater quality was within potable chemical standards. However, in contrast the results for total coliforms, *Escherichia coli* and the HPC (heterotrophic plate counts) fluctuated throughout the study period (March to August 2012), and with the exception of a few samples collected, overall the results recorded significantly exceeded ($p < 0.05$) the stipulated guidelines (DWAF, 1996; NHMRC and NRMMC, 2011). In addition, for the duration of this study, only 7.9% and 37.9% of the samples did not adhere to the guidelines for enterococci and faecal coliforms, respectively (DWAF, 1996; NHMRC and NRMMC, 2011). The total rainfall measured in sampling five to eight (ranging from 74.7 mm in June to 198.1 mm in August) was higher than the total rainfall measured in samplings one to four (ranging from 16.8 mm in March to 30.6 mm in May). However, in general due to fluctuation in indicator bacterial numbers throughout the study period, no correlation between indicator group counts and rainfall patterns could be obtained (Chapter two).

As outlined in Chapter three, phylogenetic analysis of the representative isolates for samplings one, three and six (majority of the *E. coli* strains were isolated during these sampling times) were analysed. Of particular concern was the fact that Enterotoxigenic *E. coli* (ETEC) strains, which contains the heat stable toxin (ST1), were identified predominantly in sampling one and three. Six percent of the total *E. coli* isolates were also identified as *E. coli* O157:H7 (sampling two and three). By screening the harvested rainwater samples utilising PCR assays for the presence of virulence genes specific to certain pathogenic *E. coli* strains, the presence of Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), and Enteroaggregative *E.coli* (EHEC) was also confirmed. The faeces of warm-blooded animals could serve as a possible source of *E. coli* contamination in the rainwater tanks, as warm blooded animals have been shown to carry a high number of pathogenic *E. coli* strains in their intestines.

During the high rainfall period [sampling sessions five to eight; in the article (Chapter four) subsequently referred to as sampling sessions one to four] dominant, indigenous, culturable bacteria as well as previously documented pathogens associated with DRWH were identified. The majority of the isolates were obtained during sampling one (51%) and two (27%) and amplification of the 16S rRNA region utilising PCR, showed that the dominant genera isolated were *Pseudomonas* (19%) and *Aeromonas* (16%). According to literature, many opportunistic pathogens and human pathogenic species are associated with rainwater and in the current study species associated with the genera, *Klebsiella*, *Enterobacter*, *Salmonella*, *Yersinia* and *Serratia*, amongst many others, were isolated from the harvested rainwater samples collected at the Kleinmond Housing Scheme, using genus specific primers analysis of whole DNA obtained from the rainwater. This is the first time, to the author's knowledge, that *Yersinia* spp. have been detected in harvested rainwater. This study also indicated that using one PCR assay for the detection of particular genus could indicate the presence or absence of another particular genus in harvested rainwater. For example the PCR assay used to detect the presence or absence of *Salmonella* spp. could indicate the presence or absence of *Aeromonas* spp.

Based on the microbial counts obtained on average for all the indicator organisms, and the isolation and identification of various pathogenic bacteria, harvested rainwater, that has been stored in polyethylene tanks for a short period of time (> 1 year), is not suitable for drinking purposes as per standards stipulated by the DWAF (1996) and the ADWG (NHMRC and NRMMC, 2011). Prior treatment of the rainwater is therefore required before the water source can be utilised for drinking purposes. The same observations were made for the rainwater in Hammanskraal in South Africa, where Nevondo and Cloete (1999) deemed the general quality of rainwater to be unacceptable.

Studies, world-wide, have also concluded that harvested rainwater is not suitable for drinking purposes without prior treatment (Yaziz et al., 1989; Zhu et al., 2004; Sazakli et al., 2007).

The focus of phase two was then to monitor the effectiveness of treatment systems in reducing the microbiological load of harvested rainwater. For this, three rainwater harvesting tanks were installed at the Welgevallen Experimental farm, Stellenbosch, South Africa. Although the chemical parameters of the rainwater samples collected from the Kleinmond housing scheme were all within guidelines, the fact that a different catchment area, influenced by many new variables for example, the roof used at the Welgevallen experimental farm was made of galvanised iron instead of tiles (Kleinmond Housing Scheme), meant that chemical parameters could possibly change and were therefore included.

Firstly, four different systems of filtration were intermittently connected to the rainwater harvesting tanks installed at the Welgevallen Experimental farm, including two biological filtration systems, namely slow sand filtration and slow activated carbon filtration and two microfiltration systems, namely a polyvinyl (alcohol) (PVA) nanofibre membrane column with activated carbon and then the same system was tested without activated carbon (Chapter five).

Overall the results obtained for the chemical analysis of the slow activated carbon filtration system and the slow sand filtration system after biofilm formation indicated that the rainwater quality of unfiltered and filtered rainwater samples was within potable chemical standards in the filtered rainwater samples collected on the last day from each system. The total coliforms, *E. coli* and heterotrophic bacteria counts for all unfiltered and filtered rainwater samples using the slow activated carbon filtration systems and the slow sand filtration systems exceeded the guidelines stipulated by SANS 241 (2005), the ADWG (NHMRC and NRMMC, 2011) and DWAF (1996). Moreover, genus specific PCR based assays confirmed the presence of predominantly *Yersinia* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Legionella* spp. in all unfiltered and in the filtrate of the slow sand filtration systems. Many studies have indicated that the biofilm used in biological filtration systems requires an extended time period to mature before it can provide any form of filtration. It is therefore recommended that the biological filtration systems be analysed further for a longer time period to monitor the effectiveness of a mature biofilm in the removal of pathogenic bacteria and certain contaminating chemicals.

All cations and anions monitored in the rainwater samples collected before and after filtration through the activated carbon and nanofibre column were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO

(2011). While the chemical quality of the rainwater was generally lower than the stipulated drinking water guidelines, the microbial quality of rainwater filtered through the PVA nanofibre system (without activated carbon) was inadequate according to drinking water guidelines stipulated by SANS 241 (2005), DWAF (1996) and the ADWG (NHMRC and NRMMC, 2011) and could not be used for potable purposes. The addition of activated carbon to the PVA system may have provided an additional filtration barrier, but according to the microbial analysis performed, the system including the PVA nanofibre membrane and activated carbon could only be used for the filtration of one litre of rainwater before the components in the system need to be replaced. This was confirmed with the use of PCR based assays, as *Aeromonas* spp., *Klebsiella* spp. *Legionella* spp., *Pseudomonas* spp. and *Yersinia* spp. were detected in up five litres of filtered rainwater. Moreover, the average time required to filter one litre of rainwater through the PVA nanofibre system (without activated carbon) was approximately 37.48 minutes. The addition of activated carbon to the PVA nanofibre membrane filtration system then increased the filtration time, as the average time required to filter one litre of rainwater was 44.53 minutes.

The efficiency of a solar pasteurization system in treating harvested rainwater was also investigated. Before the pasteurization pilot study, preliminary pasteurization experiments were performed in the laboratory and results indicated that overall, R2A agar was the most suited growth media for determining the recovery of heterotrophic bacteria from heat treated rainwater samples and was therefore used to assess the efficiency of the solar pasteurization system in the pilot scale study.

The solar pasteurization system was connected to one of the rainwater harvesting tanks that had been installed at the Welgevallen Experimental farm. Chemical analysis indicated that all cations and anions present in both the unpasteurized and pasteurized water were within the respective drinking water guidelines with the exception of iron, aluminium, lead and nickel. These four cations were shown to increase significantly after pasteurization at various temperatures and it is hypothesised that these elements could have leached from the stainless steel storage tanks of the pasteurization system. It is therefore recommended that the storage tank, of the pasteurization system, be manufactured from an alternative material such as Safrene^{TM} (a high density based polyethylene) that will not allow for leaching metals into the rainwater at high temperatures. Future studies should thus investigate the effect of pasteurization on the chemical quality of rainwater using a pasteurization system that will not corrode during high temperature exposure.

Microbial analysis indicated that rainwater samples pasteurized at 72°C and above (78 – 81ºC and 90 – 91ºC) could be utilised for potable purposes as total coliforms, *E. coli* and HPC were reduced to zero. However, PCR analysis of the pasteurized rainwater samples for the presence of pathogenic

bacteria at the various temperature ranges showed that *Legionella* spp. and *Pseudomonas* spp. persisted even at the temperature range of 90 to 91ºC. Although molecular techniques confirmed the presence of DNA of certain pathogenic bacteria, further studies are required to confirm the viability of specific bacterial pathogens at higher pasteurization temperatures. For this reason further studies are required to quantify and confirm the presence of viable pathogenic organisms in rainwater samples treated at temperatures greater than 72ºC.

The prospect of implementing rainwater harvesting in South Africa on a national scale has many appealing advantages. Based on the results obtained in the current study it is therefore recommended that the installation of the rainwater harvesting tanks be accompanied by the installation of a solar pasteurization system (that does not corrode) as a treatment system to provide potable water and clean water for other domestic purposes. It was also determined that the solar pasteurization system could produce an average of 61.8 L/m^2 and 0.6 L/m^2 for each kWh of incident solar energy for temperatures of 56 to 58˚C and 93 to 97˚C, respectively. Therefore throughout the year, including the winter months, solar pasteurization could provide adequate volumes of treated rainwater for drinking and domestic purposes. In agreement with Mwenge Kahinda et al. (2007) the type and capacity of the domestic rainwater harvesting system to be installed should however, depend on each community in each province of South Africa, as variables such as winter or summer rainfall could affect the managing of the treatment systems.

7.2 References

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Appendix A

Table 6.1. PCR assays for the detection of various commonly found bacteria genera detected in untreated and pasteurized rainwater samples

Sample temperature (˚C)		Organism						
		***Aeromonas* spp.**	***Klebsiella* spp.**	***Legionella* spp.**	***Pseudomonas* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Yersinia* spp.**
55	B	-	+	+	+	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	+	-	+	+
56	B	+	+	+	-	-	+	-
	A1	-	-	+	-	-	-	-
	A2	-	-	+	-	-	-	-
57	B	+	+	+	+	-	-	+
	A1	-	+	+	+	-	-	-
	A2	-	-	+	-	-	-	-
64	B	-	+	+	+	-	-	-
	A1	-	+	+	+	-	-	-
	A2	-	+	+	+	-	-	-
65	B	+	+	+	+	-	-	+
	A1	-	+	+	-	-	-	+
	A2	-	-	+	-	-	-	-
66	B	-	-	-	+	-	-	-
	A1	-	-	+	-	-	-	-
	A2	-	-	+	-	-	-	-
72	B	-	-	+	-	-	-	-
	A1	-	-	+	-	-	-	-
	A2	-	-	+	-	-	-	-
73	B	-	-	+	+	-	-	+
	A1	-	+	+	+	-	-	-
	A2	-	-	+	+	-	-	-
74	B	-	-	+	-	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	-	-	-	-
78	B	-	-	-	-	-	-	-
	A1	-	-	+	-	-	-	+
	A2	-	-	+	-	-	-	+
81	B	-	+	+	+	-	-	+
	A1	-	-	+	-	-	-	-
	A2	-	-	+	-	-	-	-
81[2nd]	B	-	-	+	+	-	-	-
	A1	-	-	+	-	-	-	-
	A2	-	-	+	+	-	-	-

90	B	-	-	+	-	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	+	-	-	-
91	B	-	+	+	+	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	+	-	-	-
91^{2nd}	B	-	-	+	+	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	-	-	-	-

B = unpasteurized rainwater

A1 & A 2 = duplicate pasteurized rainwater samples